Structure and dynamics of fluorophore-labelled DNA helices probed by NMR-spectroscopy

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To my wife

Your love and support wrote this thesis

Abstract

Structural and dynamic perturbations in DNA upon incorporation of either fluorophore, 2-Aminopurine (2AP) or 2-Hydroxy-7-nitrofluorene (HNF), are characterized by NMR spectroscopy. For this purpose the NMR solution structures of the modified DNA duplexes with the sequence 5'-GCTGCAXACGTCG-3' are solved. For X=2AP (13mer2AP) the partner base in the complementary strand is T, while for X=HNF (13merHNF) an abasic site is introduced to avoid steric strain.

By comparing results on 13mer2AP with the corresponding unmodified DNA duplex (13merRef, X=A), any perturbation can be unambiguously assigned to 2AP incorporation. For the NMR solution structure of 13merRef and 13mer2AP small but significant changes in helical parameters are found throughout the helix. Imino proton exchange measurements reveal an extended, distributed effect of 2AP incorporation on the lifetimes of the central seven base pair. However, the reduced base pair lifetime of 2AP:T cannot fully account for the rapid water exchange observed with saturation transfer experiments in the absence of base catalyst. This indicates enhanced intrinsic catalysis. As a possible catalytic site the T O4 atom opposite 2AP is discussed, which is easily accessible through the major groove and lacks a hydrogen bonding partner within the base pair.

The overall NMR solution structure is found to be B-DNA. However the NOE cross-peaks involving the HNF residue can only be accounted for by two different orientations of the HNF inside the DNA helical stack. Their population ratio is estimated to be 1:1. Dynamical perturbation is indicated by the increased linewidth and strong upfield shift of the T residue to the 5'-side of the abasic site.

Zusammenfassung

Mittels NMR-Spektroskopie werden Störungen in Struktur und Dynamik von DNA untersucht, die durch den Einbau jeweils eines der beiden Fluorophore 2-Aminopurin (2AP) und 2-Hydroxy-7-nitrofluoren (HNF) hervorgerufen werden. Zu diesem Zweck werden die NMR-Strukturen der modifizierten Duplexe mit der Sequenz 5'-GCTGCAXACGTCG-3' berechnet. Im Fall X=2AP (13mer2AP) ist die Partnerbase im Komplementärstrang ein T, während gegenüber X=HNF (13mer-HNF) eine abasische Stelle eingeführt wird.

Durch den Vergleich der Ergebnisse zum 13mer2AP mit denjenigen des entsprechenden unmodifizierten DNA Doppelstranges (13merRef, X=A) konnte jegliche Änderung eindeutig dem Einbau von 2AP zugordnet werden. Für die NMR-Strukturen von 13merRef und 13mer2AP können kleine aber signifikante, über die gesamte Helix verteilte Strukturstörungen nachgewiesen werden. Experimente zum Iminoprotonenaustausch mit Wasser ergeben, daß der Einbau von 2AP die Basenpaarlebensdauern der 7 zentralen Basenpaare erniedrigt. Die kürzere Lebensdauer des 2AP:T Basenpaares kann jedoch nicht den schnellen Wasseraustausch im Sättigungstransfer-Experiment ohne Zugabe von Basenkatalysator erklären. Als Erklärung für diese Diskrepanz wird eine effizientere intrinsische Katalyse vermutet. Als mögliche, katalytisch aktive Stelle wird das T O4 Atom diskutiert, welches über die große Furche leicht zugänglich ist und das keine Wasserstoffbrückenbindung innerhalb des Basenpaares ausbilden kann.

Die übergeordnete Struktur des 13merHNF ist eine B-Form DNA Helix. Die NOE Kreuzpeaks zu den Protonen im HNF können jedoch nur durch zwei verschiedene Orientierungen des HNFs in der helikalen Anordnung beschrieben werden. Das Verhältnis der beiden Orientierungen untereinander wird als 1:1 abgeschätzt. Störungen in der Basenpaardynamik werden durch die höhere Linienbreite und die starke Hochfeldverschiebung des T auf der 5'-Seite ausgehend von der abasischen Stelle angedeutet.

Inhaltsverzeichnis

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1 Introduction

In 1953 Watson and Crick (WC) solved the puzzle of the deoxyribonucleic acid (DNA) structure [Watson and Crick, 1953]. Aided by the results of Chargaff [1950] and Franklin and Gosling [1953] they proposed the double helical structure. Since then the double helical arrangement of DNA has become common knowledge. But although the overall conformation had been deduced, questions remained. The idealized WC-model could not explain certain sequence-specific effects; an atomic picture was lacking.

In 1980 Dickerson and coworkers were the first to solve the crystal structure of a DNA duplex [Wing et al., 1980]. They refined the WC-model and showed that the ideal helical parameters (as theoretically predicted by Watson and Crick) are true on average but can deviate substantially dependent on sequence [Dickerson and Drew, 1981b,a, Drew et al., 1981]. Subsequently, more crystal structures of DNA sequences were solved, revealing large variations in helical parameters for B-DNA [Dickerson et al., 1982, Kopka et al., 1983, Heinemann and Alings, 1989] and giving structural insights into other conformations like A-DNA [Shakked et al., 1981, Conner et al., 1982, 1984] or Z-DNA [Drew et al., 1980, Wang et al., 1981, Drew and Dickerson, 1981, Rich et al., 1984]. A detailed atomic picture of DNA helical structure was now provided.

However it was found that crystallization can have profound effects on the conformation of DNA. In the crystalline state DNA strongly favours the A-form double helix, whereas DNA in solution occurs predominantly in the B-DNA form [Bloomfield et al., 2000]. Furthermore, the helical parameters strongly depend on the crystallization conditions [Jain and Sundaralingam, 1989, Shakked et al., 1989, Johansson et al., 2000]. Thus single crystal X-ray crystallography is of limited use when studying biological problems,

particularly those involving DNA.

Parallel to the breakthrough of Dickerson and coworkers [Wing et al., 1980] the advent of 2-dimensional techniques [Jeener et al., 1979, Kumar et al., 1980a, Macura and Ernst, 1980] extended Nuclear Magnetic Resonance Spectroscopy (NMR) methods in a way that studying large biological molecules was now feasible. First efforts centered on protein structure determination [Wagner et al., 1981, Wuthrich et al., 1982, Zuiderweg et al., 1983], but eventually application to DNA structure determination in solution followed [Hare et al., 1983, Feigon et al., 1984, Clore and Gronenborn, 1984, 1985, Hosur et al., 1986]. These early studies focused on the sequential assignment of DNA or protein resonances, the structural content however was discussed only qualitatively. With the advent of powerful computers, techniques were developed that allowed for determination of 3-dimensional structures of biomolecules with NMR structural information as restraints [Williamson et al., 1985, Zuiderweg et al., 1985].

The development and subsequent refinement of the solid-phase phosphor-amidite approach for oligonucleotide synthesis [Sinha et al., 1984, Dahl et al., 1987, Schulhof et al., 1987, Caruthers et al., 1987] allowed for relatively cheap and easy access to large quantities of nucleic acids with a defined primary sequence. This marked a breakthrough for nucleic acids research since it was now possible to vary specific base positions in a predefined sequence. Thereby studying the effect of base mismatches on the helical arrangement of the duplex was facilitated [Kalnik et al., 1988, Roongta et al., 1990, Moe and Russu, 1992]. Furthermore, a means for introduction of arbitrary artificial nucleotides at any position in the duplex was provided by automated solid-phase synthesis.

Artificial DNA double strand structures have been investigated since the late 1980s [Li et al., 1987, Evans and Levine, 1988]. Several different types of modifications have to be distinguished. These involve: backbone modification [Pieters et al., 1989, Betts et al., 1995, Nielsen et al., 2009], fluorophores covalently linked to natural bases [Krugh et al., 1989, Schwartz et al., 1997, Subramaniam et al., 2001], fluorophores substituting a natural base [Nordlund et al., 1989, Guckian et al., 1998, Engman et al., 2004] or even a base pair [Matray and Kool, 1998, Guckian et al., 2000, Smirnov et al., 2002],

and intercalators, which bind to DNA through stacking and/or electrostatic interactions [Fede et al., 1993, Spielmann et al., 1995, Davies et al., 1997]. All of the latter studies focus on the introduction of chromophores, since their fluorescent properties can be exploited for studying DNA [Wojczewski et al., 1999].

The spectroscopic properties of covalently attached DNA modifications are utilized in numerous ways. Over the past decades different strategies have been developed for detecting single nucleotide polymorphisms (SNPs). These strategies employ fluorophore-quencher systems (molecular beacons) [Tan et al., 2004], DNA-mediated electron transfer (DETEQ) [Wagenknecht, 2008] or forced intercalation probes (FIT) [Koehler et al., 2005]. Furthermore, fluorescent molecules are introduced at different locations into DNA in order to get long-range structural information by exploiting fluorescence resonance energy transfer (FRET) [Lilley and Wilson, 2000]. In addition it has been demostrated that transient absorption spectroscopy of fluorophore-modified DNA can be used to follow dynamics on the pico- to femtosecond timescale [Zewail, 2000].

Supramolecular vibrational modes of biological molecules are important for their function, and many have frequencies below $200\,\mathrm{cm}^{-1}$ or 6 THz. Examples are the primary event of vision ($60\,\mathrm{cm}^{-1}$) [Wang et al., 1994], oxygen acceptance of hemoglobin ($39\,\mathrm{cm}^{-1}$) [Klug et al., 2002], chemical reactions in myoglobin ($51\,\mathrm{cm}^{-1}$) [Austin et al., 1989], and conformational change of bacteriorhodopsin ($115\,\mathrm{cm}^{-1}$) [Xie et al., 2001]. For DNA transcription the double helix must be opened to expose the coding bases to chemical reactions. Thermal melting of double-stranded oligonucleotides is similar because it starts with a "denaturation bubble" [Prohofsky et al., 1979]. The latter is reached through collective modes between 60 and $140\,\mathrm{cm}^{-1}$ which compress and stretch the interbase H-bonds [Cocco and Monasson, 2000]. However, such resonances in the low-frequency region are difficult to detect due to mixing of the DNA modes with those of hydration water. Resolving such collective vibrational modes of a biological molecule by molecular THz spectroscopy is a new but potent application for chromophores in DNA.

Here the chromophore functions like a THz light source when its charge distribution is suddenly altered by femtosecond optical excitation $S_1 \leftarrow S_0$. The electric field around

the probe is changed instantly and acts on nearby groups with partial charges. Most of these change their nuclear position in an overdamped fashion but some may oscillate briefly. Altogether a reaction field R(t) is created which is reported by the polar probe molecule, through an emission frequency which depends on R(t). The probe molecule is therefore not only light source but also detector. A response function can be obtained which is related to the local THz absorption spectrum. In this way the low-frequency vibrational structure of biomolecules can be accessed.

Molecular THz spectroscopy should reduce inhomogeneous broadening because the perturbing electric field and the reaction field are local. Only those modes will interact which have oscillator strength in the region, at the right direction. The obvious disadvantage is the need to embed a probe molecule inside double-stranded DNA as an artificial nucleobase. The probe has to be free of internal modes which are active below $\approx 300\,\mathrm{cm}^{-1}$, since they would mix with the macromolecular dynamics to be reported. For this reason the best-studied polarity probes, coumarins [Horng et al., 1995, Zhao et al., 2005], are not eligible. Instead one must use chromophors which have been shown to report the far infrared spectrum of pure liquids such as acetonitrile [Ruthmann et al., 1998, Karunakaran et al., 2008] or water [Lustres et al., 2005]. Required is bio-organic development of suited chromophores guided by optical femtosecond spectroscopy in the condensed phase. However, excellent suitability from an optical point of view is to no avail when the helical structure is severely disrupted, since duplex features are to be probed.

Thus, for all aforementioned spectroscopic techniques which investigate DNA features, it is advantageous or even imperative to know the exact orientation of the fluorophore inside the DNA double helix. Moreover it is instructive to have information on the stacking interactions of the fluorophore with the adjacent base pairs. Finally, it can be decisive - especially for biologically motivated hybridization studies - to be able to characterize the structural and dynamic perturbation of the DNA helical structure in terms of helical parameters and base pair lifetimes.

In order to solve above question, this work utilizes NMR spectroscopy for the structure

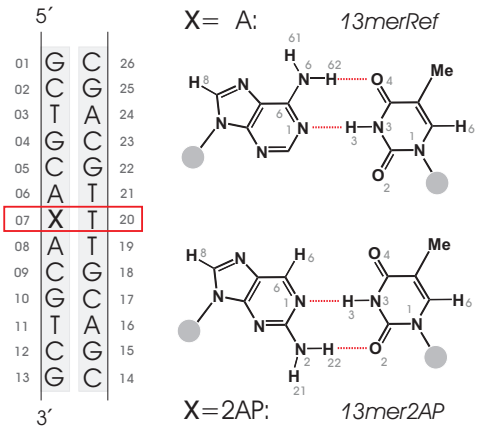


Fig. 1.1: *DNA duplex sequence with chemical structure of the 2AP-T and A-T base pairs. A symmetric, nonpalindromic 13 base pair sequence was chosen to dismiss the possibility of mispairing, loop formation or fraying effects and to have a single perturbation site.*

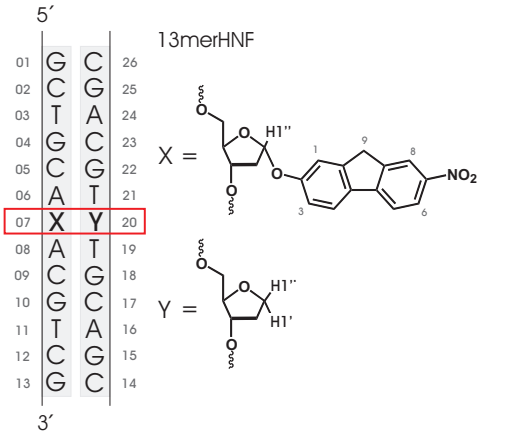


Fig. 1.2: *DNA duplex sequence with chemical structure of the HNF fluorophore. Opposite to the HNF residue has been placed an abasic site (**Y**) in order to minimize steric strain on the HNF residue.*

determination of the DNA sequences given in Fig. 1.1 and 1.2 in order to solve above question. In both sequences the central base or base pair is modified in order to have the single modification site and its adjacent base pairs unperturbed by fraying effects at the helix termini [Nonin et al., 1995]. Two different modifications are examined, 2-Aminopurine (2AP) and 2-Hydroxy-7-nitrofluorene (HNF).

Duplex DNA with 2-Hydroxy-7-nitrofluorene

The probe HNF is incorporated opposite to an abasic site (1'-2'-didesoxyribose) to avoid steric strain which might otherwise disrupt the overall B-DNA conformation or force the fluorophore into an extrahelical position. However, deletion of the partner base introduces increased flexibility into the DNA duplex at the modification site [Lin and de los Santos, 2001, Smirnov et al., 2002]. In conjunction with the different electronic properties of the HNF residue as compared to a natural base pair, HNF is expected to introduce large local perturbations compared to a more native modification. A first purpose of this work is to find out whether the DNA duplex adopts an overall B-DNA conformation

with the HNF stacked inside the double helix. This information is essential since HNF has been designed to report on macromolecular vibrational modes in DNA via transient absorption spectroscopy, which cannot be observed in case of an extrahelical orientation of HNF.

Duplex DNA with 2-Aminopurine

The 2AP-containing duplex represents a substantially different case. 2AP causes only a slight perturbation of the directly adjacent base pairs as suggested in earlier works by Lycksell et al. [1987] and Nordlund et al. [1989]. 2AP is commonly used to monitor base stacking-unstacking events in biologically relevant sequences [Allan and Reich, 1996, Reddy and Rao, 2000, Bernards et al., 2002, Daujotyte et al., 2004, Neely et al., 2005, Lenz et al., 2007]. Therefore it is crucial to characterize the perturbation induced upon 2AP incorporation structurally (in terms of helical parameters) and dynamically (in terms of reliable base pair lifetimes). This is the second aim of this work.

Since 2AP is structurally isomeric to adenine (A), it closely resembles the latter in size and shape. It has been found that it can also form stable base pairs with thymine (T) [Ronen, 1979, Sowers et al., 1986]. The two duplex structures of 13merRef with X=A and 13mer2AP with X=2AP differ only in the location of the amino group of the central residue (see Fig. 1.1). Thus any structural or dynamic differences that are observed between the two corresponding solution structures can be directly attributed to the incorporation of 2AP. This is the major difference compared to pertinent works; these investigate a ten base pair palindromic DNA duplex with two 2AP residues incorporated [Lycksell et al., 1987, Nordlund et al., 1989].

2 Theoretical background

2.1 Structural aspects of DNA

DNA is composed of four naturally occuring nucleobases, adenine (A), guanine (G), thymine (T) and cytosine (C). While A and G are derived from purine, T and C are pyrimidine derivatives. Base pairs are formed between A:T and G:C. The structure and nomenclature of these two base pair motifs is depicted in Fig. 2.1. The higher thermodynamic stability of G:C compared to A:T base pairs [Xia et al., 1998] originates from the fact that the latter base pair forms only two instead of three hydrogen bonds (Fig. 2.1).

By attaching A, G, C, or T to the C1'-atom of a 2''-deoxy-β-D-ribose the nucleosides adenosine, guanosine, cytidine and thymidine are formed, respectively. The structure and nomenclature of 2''-deoxy-β-D-ribose, which is called sugar in the following, is shown in Fig. 2.2. All atoms of the sugar are marked with a " ' " to distinguish them from nucleobase atoms. The sugar conformation is defined by five dihedral angles $\nu_0 - \nu_4$ (Fig. 2.3). The latter are interdependent and thus can be described by only two parameters

$$\nu_j = \Psi_m * \cos(P + 144(j-2)) \qquad j = 0, 1, 2, 3, 4 \tag{2.1}$$

the pucker angle P and the pucker amplitude Ψ_m. Note that this simple relation is valid only for cyclopentane, but deviations can be accounted for by introducing correction terms [Altona and Sundaralingam, 1972].

The glycosidic torsion angle χ and the backbone dihedral angles $\alpha - \zeta$ can be used to characterize the helical structure of DNA (Fig. 2.3). While $\alpha - \zeta$ define the sugar-

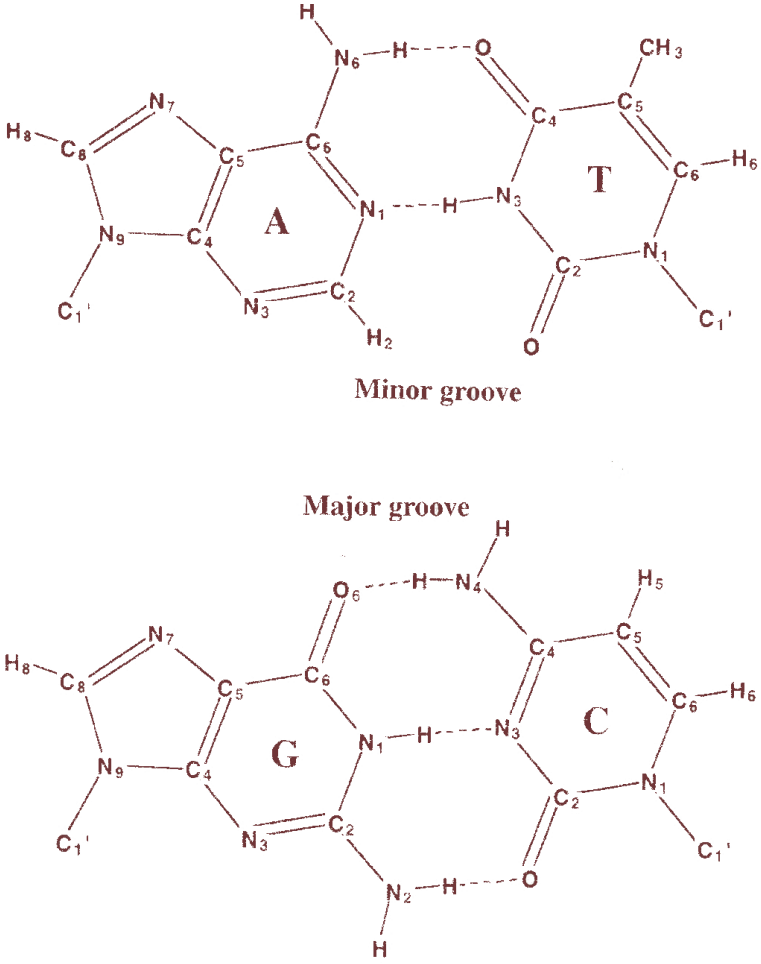


Fig. 2.1: *Structure and nomenclature of the Watson-Crick base pairs A:T and G:C. The picture is taken from Bloomfield et al. [2000].*

phosphate backbone of DNA, χ determines the position of the nucleobase relative to the sugar. Two different orientations are sampled, the anti range which centers around $\chi = -135\,^\circ$ and the syn range which samples values around $\chi = +45\,^\circ$ [Bloomfield et al., 2000]. The latter is less stable as the nucleobase is located above the sugar, which leads to steric clashes. Thus the syn-orientation of nucleobases is only found for a special helical arrangement of DNA, the left-handed Z-DNA, which needs external stabilization, e.g. by high salt concentration [Rich et al., 1984].

Two main helical arrangements are found for DNA, the A-form and the B-form. While the former is more often found in crystal structures, B-DNA is the dominant conforma-

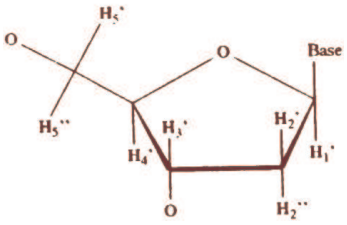


Fig. 2.2: *Nomenclature and structure of 2''-deoxy-β-D-ribose [Roberts, 1993].*

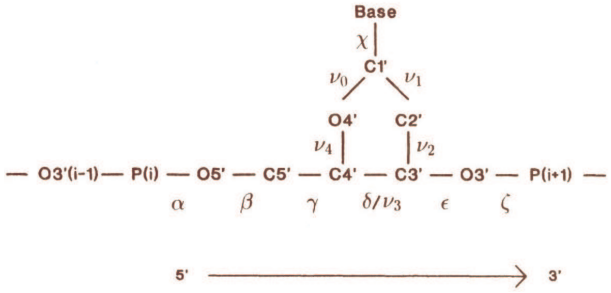


Fig. 2.3: *Dihedral angles in the sugar-phosphate backbone of DNA. The picture is taken from Roberts [1993].*

	A-DNA	B-DNA
Helix handedness	Right	Right
bp/repeating unit	1	1
bp/turn	11	10
Helix twist, (°)	32.7	36.0
Rise/bp, (Å)	2.9	3.4
Helix pitch, (Å)	32	34
Base pair inclination, (°)	12	2.4
P distance from helix axis, (Å)	9.5	9.4
X displacement from bp to helix axis, Å	−4.1	0.8
Glycosidic bond orientiation	anti	anti
Sugar conformation	C3′-*endo*	C2′-*endo*
Major groove depth	13.5	8.5
width, (Å)	2.7	11.7
Minor groove depth	2.8	7.5
width, (Å)	11.0	5.7

(a) *Important parameters*

(b) *Helical backbone*

Fig. 2.4: *Comparison of A- und B-form DNA. The pictures are taken from Bloomfield et al. [2000].*

tion in solution. The most important parameters for both helical arrangements are compiled in Fig. 2.4. While the overall arrangement is similar (right-handedness, glycosidic bond orientation), differences exist. One that is commonly used to distinguish between the two helical arrangements is the sugar conformation, with C3'-endo (P around 18 °) dominant for A-DNA and C2'-endo (P around 162 °) for B-DNA. The widths of the minor and major grooves, which are depicted on the molecular and atomic level in Fig. 2.1 and Fig. 2.4 respectively, also differ significantly. The larger helical rise and twist va-

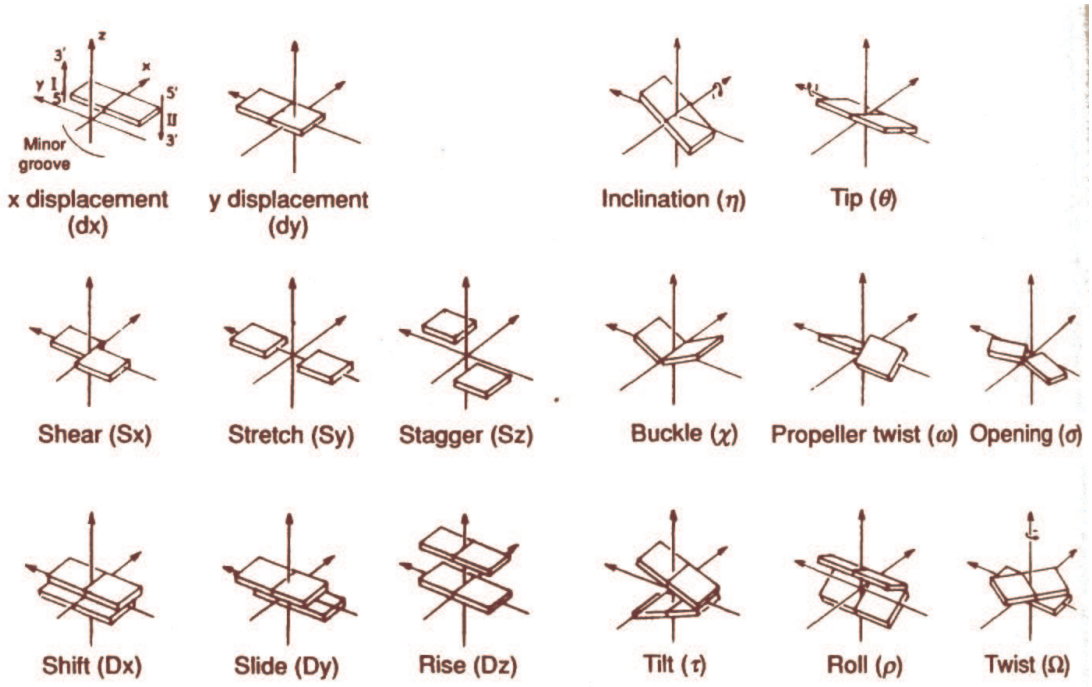


Fig. 2.5: *Helical parameters that describe the orientation of base pairs relative to the to the molecular frame (upper row), base pair partners (middle row) and conscutive base pairs (lower row) relative to each other. The picture is taken from Roberts [1993].*

lues for B-DNA lead to an elongated shape of the latter, while A-DNA is much more compressed (Fig. 2.4).

The helical parameters, which are visualized in Fig. 2.5, describe the arrangement of oligonucleotides in a double helix. They can be divided into three subgroups. Parameters in the upper row yield information on the orientation of base pairs relative to the molecular frame. The middle row parameters report on the orientation of the two base pair partners relative to each other. The lower row is most often used to characterize the helical arrangement, since these parameters give information about the orientation of two consecutive base pairs relative to each other. Especially the rise and twist-values determine the overall shape of the double helix (Fig. 2.4).

2.2 Base pair dynamics in DNA

2.2.1 Imino proton exchange theory

The imino protons of G and T are located near the helical axis at the center of each base pair and thus are effectively shielded against solvent or catalyst attack. Consequently, the central assumption in imino proton exchange theory of DNA is that exchange with bulk water can only proceed via a transient opening of the base pair [Kochoyan et al., 1987, 1988, Leijon and Graslund, 1992]. For Watson-Crick duplexes, base pair lifetimes do not depend on the nature of the adjacent pairs. This suggests that opening involves single base pairs only; the possibility of collective opening motions is ruled out [Leroy et al., 1985, Gueron et al., 1987].

Fig. 2.6 depicts a kinetic scheme of the processes involved in imino proton exchange theory [Leijon and Graslund, 1992]. Exchange from the closed state is not possible [Nonin et al., 1995], thus the first step must be the opening of the base pair with rate constants k_{op} and k_{cl} for opening and closing, respectively. From the open state, where the imino proton is assumed to be fully accessible [Kochoyan et al., 1988], two different processes can occur: exchange via an external base catalyst (rate constant k_{cat}^{ext}) and via an intrinsic pathway (k_{cat}^{int}). As a possible intrinsic catalyst the endocyclic nitrogen of the complementary base has been proposed [Leroy et al., 1985, Kochoyan et al., 1988]. Frequently used external base catalysts are Trishydroxymethylaminomethane (TRIS) or ammonia [Kochoyan et al., 1988, Moe and Russu, 1990, Bhattacharya et al., 2002].

Under conditions of a stable structure ($k_{op} << k_{cl}$), which can be safely assumed for duplex lengths > 10 base pairs, the concentration of base pairs in the open state $[NH^*]$ is quasistationary, i.e. the kinetics are pseudo-first-order.

$$\frac{d[NH^*]}{dt} = 0 = k_{op}\,[NH^* \cdots N] - (k_{cl} + k_{cat}^{int} + k_{cat}^{ext})\,[NH^*] \tag{2.2a}$$

$$(k_{cl} + k_{cat}^{int} + k_{cat}^{ext})\,[NH^*] = k_{op}\,[NH^* \cdots N] \tag{2.2b}$$

$$[NH^*] = \frac{k_{op}}{(k_{cl} + k_{cat}^{int} + k_{cat}^{ext})}\,[NH^* \cdots N] \tag{2.2c}$$

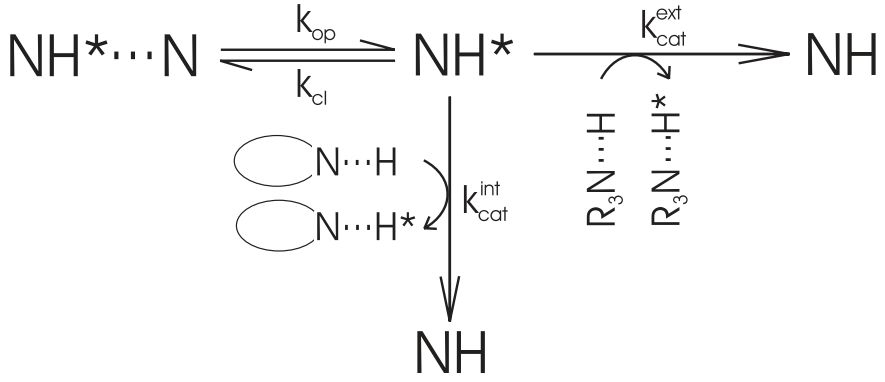


Fig. 2.6: *Kinetic scheme of imino proton exchange*

The effective imino proton exchange rate k_{ex} is defined by

$$k_{ex}[NH^* \cdots N] = (k_{cat}^{int} + k_{cat}^{ext})\,[NH^*] \tag{2.3}$$

where $[NH^* \cdots N]$ is the concentration of the base pair in the closed state. Substituting eq. (2.2c) into eq. (2.3) and cancelling of $[NH^* \cdots N]$ gives

$$k_{ex} = k_{op}\,\frac{k_{cat}^{int} + k_{cat}^{ext}}{k_{cl} + k_{cat}^{int} + k_{cat}^{ext}} \tag{2.4a}$$

$$\frac{1}{k_{ex}} = \frac{1}{k_{op}}\,(1 + \frac{k_{cl}}{k_{cat}^{int} + k_{cat}^{ext}}) \tag{2.4b}$$

which can be reduced to

$$\frac{1}{k_{ex}} = \frac{1}{k_{op}} + \frac{1}{K_{diss}\,(k_{cat}^{int} + k_{cat}^{ext})} \tag{2.5}$$

by substituting $K_{diss} = \frac{k_{op}}{k_{cl}}$. Introducing $\tau_{ex} = \frac{1}{k_{ex}}$, the imino proton exchange time, and $\tau_{op} = \frac{1}{k_{op}}$, the base pair lifetime, gives

$$\tau_{ex} = \tau_{op} + \frac{1}{k_{cat}^{int}\,K_{diss} + k_{cat}^{ext}\,K_{diss}} \tag{2.6}$$

The transfer rate of the imino proton to an external catalyst in the isolated mono-

nucleoside (k_i) should be comparable to k_{cat}^{ext}. The former is given by

$$k_i = \frac{k_{coll}\,[B]}{1 + 10^{pK_a^n - pK_a^c}} = k_{iso}\,[B] \tag{2.7}$$

where k_{coll} is the collision rate constant and pK_a^n and pK_a^c are the pK_a-values for the nucleotide and the catalyst respectively [Eigen, 1964]. Since the latter are all constant, they can be substituted by introducing k_{iso}, the constant of proportionality between k_i and the concentration of the external base catalyst [B]. Thus k_{cat}^{ext} can be expressed as

$$k_{cat}^{ext} = \alpha\, k_{iso}\,[B] \tag{2.8}$$

where restricted accessibility of the imino proton in the open state as compared to the isolated nucleoside is taken into account by the parameter α, which ranges from 0 (not accessible) to 1 (unrestricted accessibility). For natural base pairs and all the commonly used base catalysts α was found to be approximately 1 [Kochoyan et al., 1988]. Consequently, differences in accessibility might become important for modified nucleotides, but can be safely neglected for natural ones. Substituting eq. (2.8) into eq. (2.6) gives

$$\tau_{ex} = \tau_{op} + \frac{1}{k_{cat}^{int}\,K_{diss} + \alpha\, k_{iso}\,K_{diss}\,[B]} \tag{2.9}$$

which is an accurate description when $k_{cat}^{int} \approx k_{cat}^{ext}$. At high external base catalyst concentrations, k_{cat}^{ext} dominates k_{cat}^{int} and eq. (2.9) simplifies to

$$\tau_{ex} = \tau_{op} + \frac{1}{(\alpha\, k_{iso}\,K_{diss})}\,\frac{1}{[B]} \tag{2.10}$$

and the imino proton exchange time becomes a linear function of the inverse of the external catalyst concentration. In the other extreme case, when no external catalyst is available, $k_{cat}^{int} >> k_{cat}^{ext}$ and consequently eq. (2.9) reduces to

$$\tau_{ex} = \tau_{op} + \frac{1}{k_{cat}^{int}\,K_{diss}} \tag{2.11}$$

which means that changes in the imino proton exchange can be either due to different base pair lifetimes (which can be determined via eq. (2.10)) or altered intrinsic exchange.

Early works on imino proton exchange considered the opening of the base pair to be rate-limiting, i.e. $k_{cl} << (k_{cat}^{int} + k_{cat}^{ext})$ [Teitelbaum and Englander, 1975b,a, Patel and Hilbers, 1975, Hilbers and Patel, 1975]. In that case, eq. (2.4a) simplifies to

$$k_{op} \frac{k_{cat}^{int} + k_{cat}^{ext}}{k_{cat}^{int} + k_{cat}^{ext}} = k_{op} = k_{ex} \tag{2.12}$$

which implies that imino proton exchange with water directly measures the lifetime of the closed base pair (τ_{op}) since the latter is no longer a function of [B]. Exchange occurs every time the base pair opens, thus τ_{ex} and consequently τ_{op} are maximal. As a result, base pair lifetimes published before 1985 were overestimated by approximately one order of magnitude. In that year Gueron and coworkers showed that τ_{ex} depends on the external catalyst concentration [Leroy et al., 1985, Gueron et al., 1987], demonstrating that imino proton exchange in polynucleotides is not opening-limited. Instead, eq. (2.10) is validated by their results. By introducing the apparent dissociation constant $\alpha\, K_d$ (eq. (2.13a)) [Kochoyan et al., 1988] one obtains the commonly used expression for τ_{ex} (eq. (2.13b)):

$$\alpha\, K_d = \alpha\, k_{iso}\, K_{diss} \tag{2.13a}$$

$$\tau_{ex} = \tau_{op} + \frac{1}{(\alpha\, K_d)} \frac{1}{[B]} \tag{2.13b}$$

A plot of $[B]^{-1}$ vs τ_{ex} allows for determination of the apparent dissociation constant from the slope of the linear fit. However, interpretation of $\alpha\, K_d$ is not straightforward and hampered by the approximations detailed above. Much more informative of base pair dynamics is τ_{op}, which can be determined from the intercept with the ordinate (in the limit of infinite $[B]$) of the plot $[B]^{-1}$ vs τ_{ex}.

Based on the reaction $[BH^+] + [H_2O] \rightleftharpoons [B] + [H_3O^+]$ and the definition of the acidity constant (K_s)

$$K_s = K_{eq}\,[H_2O] = \frac{[B]\,[H_3O^+]}{[BH^+]} \tag{2.14}$$

an expression is obtained, which relates the concentration of free external base catalyst $[B]$ and the total added base concentration $[B_0]$.

$$\frac{1}{[B]} = (1 + 10^{(pK_a^c - pH)}) \frac{1}{[B_0]} \tag{2.15}$$

It follows that $[B]$ is a function of $[B_O]$ and the pH-value.

The imino proton exchange time τ_{ex} can be determined by measuring the line broadening of the imino proton resonance due to addition of base catalyst [Lycksell et al., 1987].

$$\frac{1}{\tau_{ex}} = \pi \, \Delta \tag{2.16}$$

Line broadening provides an easy and time-efficient way to experimentally determine τ_{ex}. However, since line broadening can also have other sources apart from the exchange process (e.g. different shim settings for each titration point) results obtained with this method tend to be inaccurate.

An alternative way is to calculate τ_{ex} from the difference of the spin-lattice relaxation time with (T_1^{ext}) and without external base catalyst (T_1^{int}) [Bhattacharya et al., 2002].

$$\frac{1}{\tau_{ex}} = \frac{1}{T_1^{ext}} - \frac{1}{T_1^{int}} \tag{2.17}$$

T_1^{ext} and T_1^{int} can be measured by NMR spectroscopy with high precision - errors below 2 % can be achieved [Sass and Ziessow, 1977] - and thus allow for more precise values of τ_{ex} to be determined.

2.2.2 Inversion recovery experiments

Base pair dynamics of DNA can be followed by NMR spectroscopy. As detailed above (section 2.2.1), the key observable is chemical exchange of imino protons with bulk water as a function of external catalyst concentration. According to eq. (2.17) τ_{ex} can be measured by determining T_1^{ext} and T_1^{int}. However, a difference in magnetization between the imino proton and water protons has to be created in order to make the exchange observable by NMR.

This difference can be created by employing two alternative experimental approaches, each of which targets either the water or the imino proton signals. Consequently, four different approaches can be distinguished in the literature: *selective saturation of the water signal* [Leroy et al., 1985, Nonin et al., 1995], *selective saturation of the imino proton region* [Lycksell et al., 1987, Kochoyan et al., 1987, 1988, Leroy et al., 1988, Moe and Russu, 1990, 1992, Leijon and Graslund, 1992, Leroy et al., 1993, Folta-Stogniew and Russu, 1994, Moe et al., 1995, Folta-Stogniew and Russu, 1996, Leijon, 1996], *selective inversion of the water signal* [Snoussi and Leroy, 2001, Mihailescu and Russu, 2001, Chen and Russu, 2004, Coman and Russu, 2005] and *selective inversion of the imino proton region* [Dornberger et al., 1999, Bhattacharya et al., 2002].

Although the majority of the former studies used the saturation recovery method, more recent works rely on inversion recovery experiments. Saturation recovery might be preferable for measurements of long T_1-values ($> 5\,\mathrm{s}$) [Levy and Peat, 1975] or when the choice of optimal delay times is difficult due to a large range of T_1-values of interest [Roscher et al., 1996]. But these conditions are clearly not fulfilled for T_1-values of imino protons which are on a ms-timescale [Moe and Russu, 1990]. Becker et al. [1980] compared the efficiency of inversion and saturation recovery in determining T_1-values with a certain precision. They report inversion recovery to be much more efficient, with saturation recovery requiring 8-times more scans to achieve comparable signal-to-noise ratio [Weiss et al., 1980, Becker et al., 1980]. Thus inversion recovery should generally be preferred over saturation recovery experiments.

As to the question whether to invert the water or the imino proton region, no com-

prehensive study has yet been made. Regarding water inversion, Mihailescu and Russu [2001] point out that in order to obtain values for τ_{ex}, the magnetization of the imino protons as a function of the exchange delay τ has to be fitted to the equation

$$M(\tau) = M(0) - \frac{[M(0) - M^0]}{(T_1 + k_{ex})^{-1}} - \frac{k_{ex}\, M_0\, T_1^{water}\, \tau}{(q-1)^{-1}} \tag{2.18}$$

where the values of T_1^{water} and q (efficiency of inversion for the water signal) have to be determined in a separate experiment. Furthermore, the value of $(T_1 + k_{ex})$ is determined for each imino proton resonance of interest by selective saturation [Mihailescu and Russu, 2001]. These additional experiments are time-consuming and thus inversion of the water signal is not to be preferred. In consequence, the method of choice for measuring base pair lifetimes is selective inversion of the imino proton region.

In the standard inversion recovery experiment the z-magnetization of one or multiple spins is inverted by an initial 180 °-pulse ($+M_z \rightarrow -M_z$). After a variable delay time (τ) a 90 °-pulse is used to detect the recovered magnetization (Fig. 2.7). The plot of signal intensity vs delay time is fitted exponentially with three instead of two parameters (A,B and T_1) to account for imperfect inversion of the signal

$$I(\tau) = A + B\, exp(-\tau/T_1) \tag{2.19}$$

[Sass and Ziessow, 1977, Kowalewski et al., 1977]. To create differences in magnetization between water and the imino protons, the initial 180 °-pulse has to be applied selectively to the imino proton region.

Selective inversion can be achieved in numerous ways. In principle, the commonly used constant-amplitude (rectangular), unselective (hard) pulse can be applied selectively by varying the pulse length and adjusting the pulse power. Rectangular pulses have the advantage of relatively straightforward implementation and optimization due to their analytical solution to the Bloch equations [Hajduk et al., 1993]. However, the excitation profile, which can be calculated by the latter equations, is imperfect. While there is a central lobe of strong excitation, the sharp leading and trailing edges of the pulse give rise

to a set of sidelobes (cf. Fig. 2.8), whose amplitude decreases with offset from the centre frequency [Freeman, 1992]. The sidelobes lead to considerable off-resonance excitation. Similar reasoning can be applied to inversion. In order to reduce off-resonance excitation, the transition region at the edges of the pulse must be smoothed.

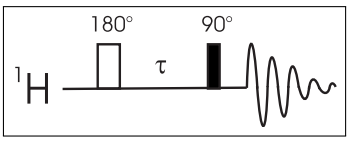


Fig. 2.7: *Scheme of a general inversion recovery pulse sequence*

A number of shaped pulses for selective bandwidth inversion were created when - with the advent of the computer - numerical calculation of the Bloch equations became possible. Among the first shapes to be proposed was the Gaussian pulse envelope, which is still popular [Bhattacharya et al., 2002]. The advantage of the latter is that in the linear response region (pulse length $\tau_p << T_1, T_2$) the excitation function is another Gaussian (see Fig. 2.8). Thus sidelobes are effectively avoided [Bauer et al., 1984]. However, a Gaussian excitation function is far from the ideal of a rectangle, consequently the completely inverted region is quite small [McDonald and Warren, 1991]. Other pulse shapes like Hermitian [Warren, 1984], Gaussian cascades [Emsley and Bodenhausen, 1990] and quaternion pulses [Emsley and Bodenhausen, 1992] try to extend the region of complete inversion with increasing success.

However, the advantages, which were outlined above are achieved at the cost of longer pulse durations. But when the the relaxation times T_1 and T_2 become comparable to (or even shorter than) the pulse duration, relaxation during the 180 °-pulse is no longer negligible. This can have a profound effect on the excitation profile and hamper applicability of shaped pulses for selective excitation [Hajduk et al., 1993]. Furthermore, most shaped pulses were derived on the basis of the Bloch equations neglecting such effects as radiation damping, relaxation and coupling [McDonald and Warren, 1991]. While each of these effects can be accounted for separately [Warren et al., 1989, Hajduk et al., 1993, Ewing et al., 1990], to account for all of them at once is difficult.

The adiabatic sweep is a completely different approach to selective bandwidth inversion. The usually employed pulsed NMR experiment is operated at a static magnetic field B_o and uses a pulsed magnetic field B_1 to simultaneously invert all frequencies of

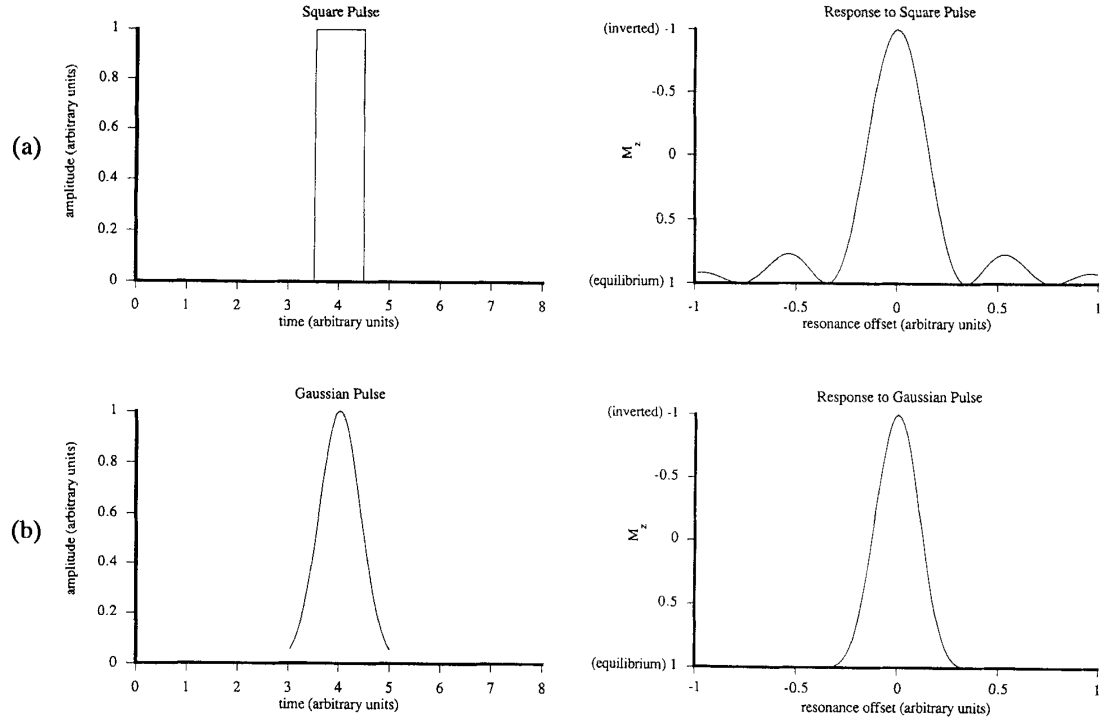


Fig. 2.8: *Pulse shapes and excitation forms of a) rectangular and b) Gaussian pulse. This picture is taken from McDonald and Warren [1991].*

interest with a constant, bandwidth-centered carrier frequency. In the adiabatic passage experiment, the carrier frequency is modulated over the whole bandwidth and thus the frequencies of interest are inverted successively [Tannús and Garwood, 1997]. An alternative, equivalent approach is to modulate the phase of the center frequency [Garwood and DelaBarre, 2001]. Adiabatic pulses have two main advantages over standard pulses. They are rather insensitive to B_1 field inhomogeneity [Bohlen and Bodenhausen, 1993] and require much less radiofrequency power for inverting nuclear spins over a wide range of chemical shifts [Kupce and Freeman, 1995]. Many different pulse shapes, which enhance one or the other advantage, were created: the hyperbolic secant pulse [Silver et al., 1984, 1985], the chirped pulse [Bohlen and Bodenhausen, 1993, Fu and Bodenhausen, 1995] or the WURST pulse [Kupce and Freeman, 1995, 1997]. Some are now utilized in broadband heteronuclear spin decoupling where radiofrequency power is limited [Kupce and Freeman, 2007] or in *in vivo* NMR where surface coils with a spatially inhomoge-

neous B_1 field are used [Tannús and Garwood, 1997]. Yet there are also disadvantages. One of them is a distinct phase distortion which is introduced for large bandwidths due to the different times at which the frequencies are inverted. This disadvantage can be circumvented, however, by introducing a second adiabatic pulse which cancels the phase distortion introduced by the first [Kupce and Freeman, 1997]. Further disadvantages are the requirement that adiabatic rotations must be accomplished rapidly relative to T_1 and T_2 of the nuclear spins of interest [Garwood and DelaBarre, 2001]. This limits application of adiabatic pulses to spins with quite long relaxation times and those which span a large number of chemical shifts, e.g. heteronuclei like ^{13}C.

2.3 Solution structure determination of DNA

2.3.1 Nuclear Overhauser Effect spectroscopy

NMR spectroscopy has become the method of choice for structure determination of biomolecules in solution. This success is primarily based on the possibility to directly extract distance information utilizing the Nuclear Overhauser Enhancement effect (NOE). Overhauser [1953] was the first to discover that the intensity of the signal of one resonance is changed upon perturbation of another due to cross-relaxation. Initially the NOE was observed by selective saturation of one line and subsequent recording of the 1D spectrum over the whole spectral region of interest. This approach proved to be very useful for the structure elucidation of small molecules [Colson et al., 1967, Woods et al., 1968, Schirmer et al., 1970, Schirmer and Noggle, 1972]. Its applicability to biomolecules however, was limited by the long accumulation time and severe spectral overlap which leads to poor selectivity of saturation [Kumar et al., 1981]. The development of the 2D-NMR spectroscopy finally allowed to aquire the complete set of NOE effects for a macromolecule with a single experiment [Macura and Ernst, 1980]. Furthermore, the 2D Nuclear Overhauer Enhancement Spectroscopy (NOESY)-experiment could be readily repeated in H_2O [Kumar et al., 1980b].

The pulse sequence of a general 2D NOESY experiment is shown in Fig. 2.9. With an initial 90 °-pulse transverse magnetization is created. The latter is allowed to precess freely during the evolution time t_1, thereby frequency-labelling the magnetization components. A second 90 °-pulse rotates the magnetization onto the negative z-axis. During the subsequent mixing period with variable length τ_m, z-magnetization components exchange through dipole-dipole cross-relaxation. A third 90 °-pulse again creates transverse magnetization which is finally detected. All three pulses are applied non-selectively [Macura and Ernst, 1980].

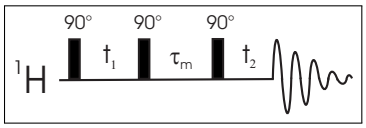


Fig. 2.9: *Scheme of a general NOESY pulse sequence*

In the absence of scalar spin-spin-interactions, cross-relaxation of the longitudinal

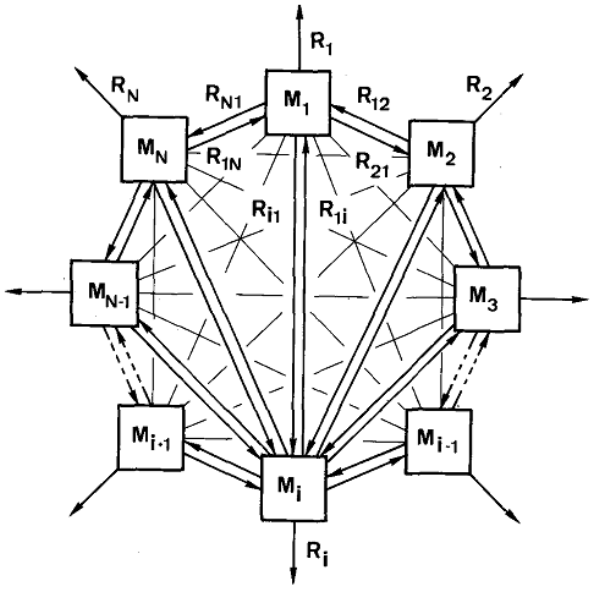


Fig. 2.10: *The cross-relaxation network of N groups of nuclei. The cross relaxation rates R_{ij} lead to a distribution of magnetization within the system, while the leakage relaxation rates R_i lead to a loss of magnetization towards the environment. This picture is taken from Macura and Ernst [1980].*

magnetization components M_{ij} can be described with the equation:

$$\dot{\mathbf{m}} = \mathbf{R} \times \mathbf{m} \tag{2.20}$$

$\mathbf{R}$ is the relaxation matrix comprising the cross relaxation rates R_{ij} and the external relaxation (leakage) rates R_i (Fig. 2.10). The vector $\mathbf{m}$ comprises the deviation of M_{zi} from thermal equilibrium for i spins

$$m_i = M_{zi} - \frac{n_i}{N} M_o \tag{2.21}$$

where M_o is the total equilibrium magnetization of the N nuclei. After the evolution period the initial z-magnetization components are encoded by the precession frequencies

of the components (ω_i)

$$m_i(0) = M_o \frac{n_i}{N} \left[cos(\omega_i t_1) \, exp \left(- \frac{t_1}{T_{2i}} \right) - 1 \right] \tag{2.22}$$

such that the cross-relaxation pathway can be traced back to its origin. The recovery of the magnetization back to equilibrium during the mixing period can be described by the following solution to eq. (2.20) [Macura and Ernst, 1980]

$$\mathbf{m}(\tau_m) = exp[-\mathbf{R}\,\tau_m]\,\mathbf{m}(0) \tag{2.23}$$

where $\mathbf{m}(\tau_m)$ is the matrix comprising the magnetization components after the mixing period, $\mathbf{m}(0)$ represents the intensities of the diagonal peaks at $\tau_m = 0$. The diagonal (R_{ii}) and off-diagonal (R_{ij}) relaxation matrix elements are given as [Roberts, 1993]:

$$R_{ii} = q_{ij} \sum_{i,j} \Big\{ J_{0,ij}(\omega_i - \omega_j) + 3\,[J_{1,ij}(\omega_i) + J_{1,ij}(\omega_j)] + 6\,J_{2,ij}(\omega_i + \omega_j) + R_{1i} \Big\} \tag{2.24a}$$

$$R_{ij} = q_{ij}\,[6\,J_{2,ij}(\omega) - J_{0,ij}(\omega)] \tag{2.24b}$$

where R_{1i} is the leakage rate, which can usually be neglected in the absence of paramagnetic nuclei. The factor q_{ij} comprises all constant values

$$q_{ij} = \frac{\gamma_i^2\,\gamma_j^2\,\hbar^2\,\mu_0^2}{160\,\pi^2} \tag{2.25}$$

where γ_i and γ_j are the gyromagnetic ratios for spins i and j respectively, $\hbar$ is the Planck constant (divided by 2π) and μ_0 is the magnetic constant or vacuum permeablility. $J_{n,ij}(\omega)$ represents the spectral densities for the zero, single and double quantum transitions (n=0,1,2 resp.)

$$J_{n,ij}(\omega) = \frac{\tau_c^{ij}}{1 + \left(n\,\omega_0\,\tau_c^{ij}\right)^2} \frac{1}{r_{ij}^6} \tag{2.26}$$

where τ_c^{ij} is the rotational correlation time of the vector between spins i and j and r_{ij} is the distance between the latter. Since the differences in resonance frequency for various spins are negligibly small compared to the value of the resonance frequency itself, ω_i and ω_j are approximated by the center frequency ω_0. The factor n in the denominator is given by the number of quanta involved in the transition. With the help of some assumptions the intensities of NOE cross-peaks can be directly related to distances in the molecule under investigation, as is seen in the next section.

2.3.2 Structural information from Nuclear Overhauser Enhancement effects

The NOE effect is an invaluable tool for structure elucidation, since it is correlated with the distance between the interacting nuclei. There are different methods to extract structural information out of NOE cross-peak intensities. A very popular method is to classify peaks according to their peak intensities into strong, medium and weak and setting up loose distance restraints of 1.8-2.8 Å, 1.8-3.3 Å and 1.8-5.0 Å, respectively. This approach has the clear advantage of simplicity and abolishes the need to integrate NOE cross-peaks in the 2D-spectrum. However, intensities are only representative of peak volumes when the lineshape for all peaks is the same.This is true only when the digital resolution is larger than the peakwidth [Roberts, 1993], which is clearly not fulfilled for modern high-resolution spectra of macromolecules. A second disadvantage is that such loose distance restraints do not have much restraining power and consequently the structure is ill-defined. In particular for nucleic acids, the classification on the basis of peak intensities is unsuitable, since the proton density is much less than in proteins (0.35 protons/atom versus 0.52 protons/atom in proteins) and thus yields fewer distance restraints. Additionally, long-range peaks as detected in proteins cannot be observed in DNA due to its rod-like shape. The need for more accurate distance restraints in DNA structure determination by NMR is thus intensified [MacDonald and Lu, 2002].

Very accurate distance restraints can be extracted using the Full Matrix Relaxation Approach. Here distances are calculated directly from the off-diagonal elements of the relaxation matrix, which in turn is obtained via

$$\mathbf{R} = \frac{ln[\mathbf{m}(0)] - ln[\mathbf{m}(\tau_m)]}{\tau_m} \tag{2.27}$$

[Roberts, 1993]. To obtain the complete intensity matrix $\mathbf{m}$ is not possible in practice. Thus two different algorithms have been proposed to fill the "gaps" in $\mathbf{m}$. Schematic representations for both algorithms are given in Fig. 2.11. Both algorithms rely on a reasonable starting structure from which an intensity matrix is calculated. The latter is then combined with the intensity matrix derived from the experimental NOEs and

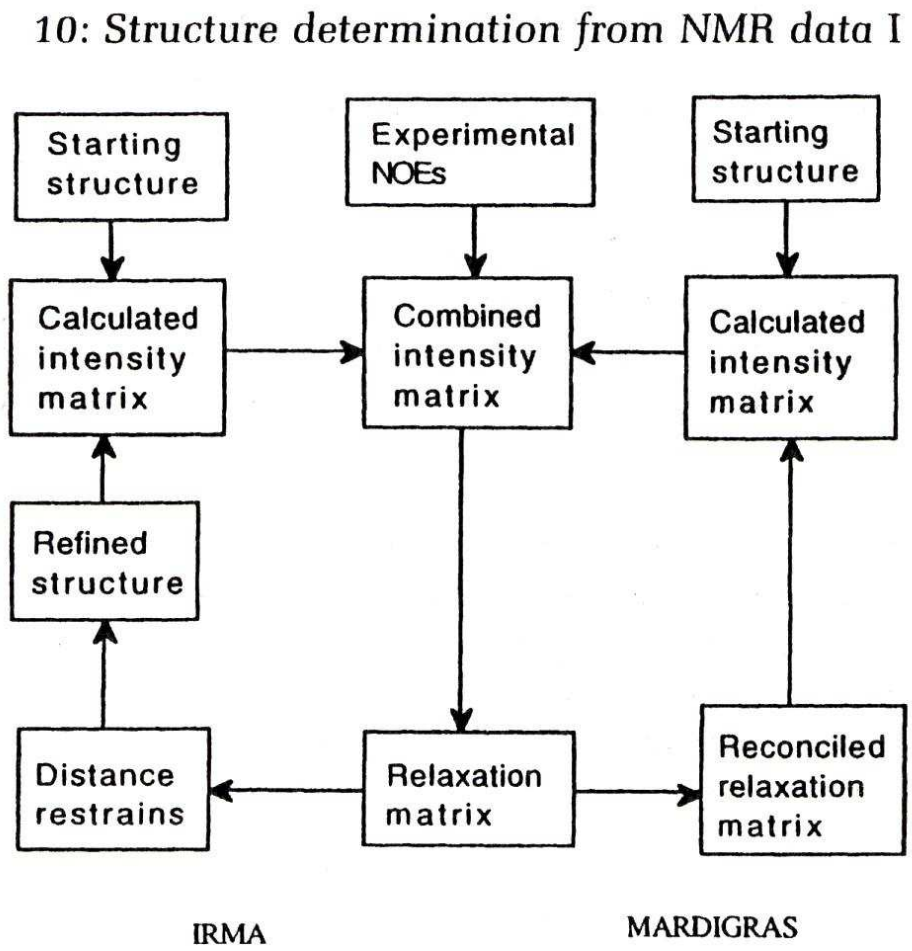


Fig. 2.11: *Two different approaches to obtain distances from NOE experiments. While one approach is based on the self-consistency of the relaxation matrix (right part), the other relies on external structure calculation (left part). Popular implementations of these approaches are the programs MARDIGRAS [Borgias and James, 1990] and IRMA [Boelens et al., 1988]. This picture is taken from Roberts [1993].*

the relaxation matrix is calculated using eq. (2.27). At this step the two algorithms differ. One approach checks the relaxation matrix for self-consistency and produces a reconciled relaxation matrix (MARDIGRAS [Borgias and James, 1990]) from which the intensity matrix is in turn calculated. The other approach (IRMA [Boelens et al., 1988]) produces a set of distance restraints from the relaxation matrix. The restraints are used to refine the starting structure. The refined structure is then used to calculate an intensity matrix and the process starts all over again. The main advantage of either approach

is that by measuring NOESY spectra at different mixing times, spin diffusion can be accounted for and very accurate distance restraints are produced. However, as pointed out by Lane [1996] and Tonelli and James [1998] error limits are often too small since conformational averaging leads to considerable errors in NOE intensiy. Furthermore, aquiring and especially accurately integrating NOE data for several mixing times is exceedingly time-consuming.

A third possibility to derive distance restraints from NOE cross-peak intensities is the Isolated Spin Pair Approximation (ISPA). Several assumptions are made. First, a single correlation time (τ_c) for the whole molecule is introduced, with which τ_c^{ij} in eq. (2.26) is replaced.

$$J_{n,ij}(\omega) = \frac{\tau_c}{1 + n^2\,\omega_0^2\,\tau_c^2}\,\frac{1}{r_{ij}^6} \tag{2.28}$$

In some cases local mobility of residues must be taken into account and a modified spectral density function has to be used [Lipari and Szabo, 1982a,b]

$$J_{n,ij}(\omega) = \left(\frac{S^2\,\tau_c}{1 + n\,\omega_0^2\,\tau_c^2} + \frac{S^2\,\tau_e}{1 + n\,\omega_0^2\,\tau_e^2}\right)\frac{1}{2\,r_{ij}^6} \tag{2.29}$$

where τ_e is the effective correlation time of the local mobility site and S^2 is the generalized order parameter, which is a measure for the flexibility of the site with values ranging from 0 (unrestricted motion) to 1 (fully restricted motion). In proteins, order parameters range from 1 to as low as 0.6 for flexible side chains [Flynn et al., 2001], while S^2 in DNA is on the order of 0.8 for all proton pairs [Lane, 1993, 1996]. In the ISPA approach all distances are referenced to a fixed distance (both of which have $S^2 \approx 0.8$). Thus the contribution of local mobility is cancelled. The assumption of a single correlation time for the whole molecule is valid since correlation times for base and sugar protons are comparable [Reid et al., 1989] and oligonucleotides shorter 15 base pairs in length can be assumed isotropic rotors [Birchall and Lane, 1990].

In the ISPA approach the matrix exponential of eq. (2.23) is approximated with a Taylor expansion

$$exp[-\mathbf{R}\,\tau_m] = 1 - \mathbf{R}\,\tau_m + \frac{1}{2}\mathbf{R}^2\,\tau_m^2 - \ldots \tag{2.30}$$

whereby the peak intensities in the NOESY spectrum are given as

$$A_{ij} = \delta_{ij} - R_{ij}\,\tau_m + \frac{1}{2}\sum_k R_{ik}\,R_{jk}\,\tau_m^2 - \ldots \tag{2.31}$$

The central assumption in ISPA is that for short mixing times, the Taylor expansion can be truncated after the linear term. Thus any effects of spin diffusion, which is magnetization transfer over third atoms (represented by the quadratic term), are neglected and the cross-peak intensity ($i \neq j$) becomes a linear function of r_{ij}^{-6}.

$$A_{ij} = R_{ij}\,\tau_m = q_{ij}\,\tau_c\,\tau_m \left(\frac{5 - 4\,\omega_0^2\,\tau_c^2}{1 + 4\,\omega_0^2\,\tau_c^2}\right)\frac{1}{r_{ij}^6} \tag{2.32}$$

Consequently, distances can be derived by referencing to a known, fixed distance whereby all constant terms are cancelled.

$$\frac{A_{ref}}{A_{ij}} = \left(\frac{r_{ij}}{r_{ref}}\right)^6 \qquad or \qquad r_{ij} = r_{ref}\,\sqrt[6]{\frac{A_{ref}}{A_{ij}}} \tag{2.33}$$

A commonly used reference distance is the C H5-H6 distance which is fixed at appr. 2.5 Å [Reid et al., 1989]. While many cross-peaks can be referenced with this distance, it is necessary to introduce several others to account for fast rotation in methyl groups (e.g. C7-H7) or solvent exchange with amino and imino protons (e.g. H42-H5).

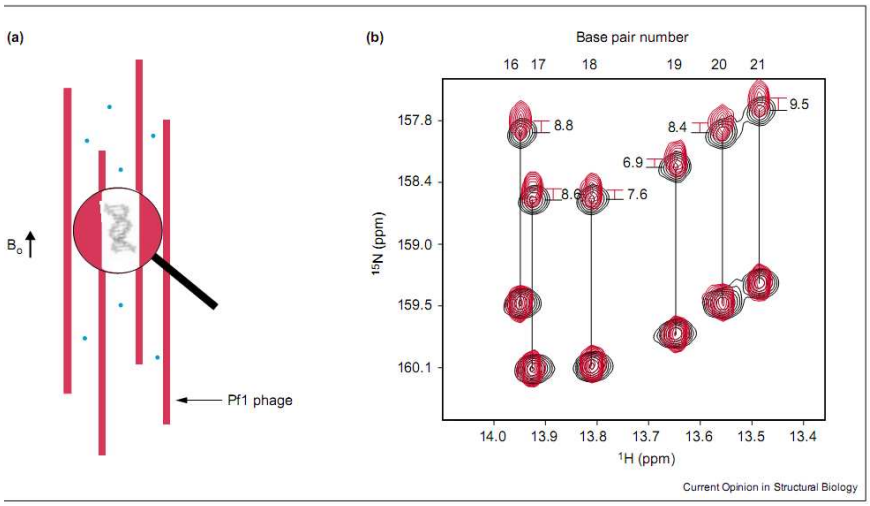


Fig. 2.12: *Panel (a) shows the steric interaction of DNA with Pf1 which prevents the DNA from tumbling isotropically and thus induces residual order. The latter gives rise to Residual Dipolar Couplings (RDC) which can be determined by measuring the difference in dipolar coupling of i.e. an N-H bond vector with and without alignment (panel (b)). This picture is taken from MacDonald and Lu [2002].*

2.3.3 Residual Dipolar Couplings

In combination with NOE data, Residual Dipolar Couplings (RDC) are now routinely employed for the NMR structure determination of macromolecules as they compensate for the drawbacks of NOE data. Due to the r_{ij}^{-6}-dependence of the NOE (cf. section 2.3.1) only information on closely spaced spins ($r_{ij} \approx 5\,\text{Å}$) is provided. In proteins, long-range peaks between residues far apart in the primary sequence can be observed owing to the tertiary fold. Due to the rod-like shape of short and medium-size DNA, only information on directly adjacent base pairs is available. Long-range effects like kinking in A-tract DNA could thus not be described prior to the development of RDC measurement [MacDonald et al., 2001, Stefl et al., 2004].

RDCs yield information on the orientation of bond vectors relative to the molecular frame. Thus also distant parts in a macromolecule can be characterized relative to each other, which significantly improves results from structure calculations, especially with regard to the global fold [Mauffret et al., 2002].

The first account of measuring anisotropic interactions was published more than 45

years ago by Saupe and Englert [1963]. Only after the advent of high-resolution NMR spectrometers and methods, this technique could be applied to biological macromolecules with success. Bax and Tjandra [1997] were able to demonstrate that dissolving the protein ubiquitin in a very dilute solution of bicelles induces residual order while retaining the high resolution of NMR spectra. This marked a breakthrough in the field of biomolecular NMR since measurement of residual order effects such as RDCs, were not limited to molecules with natively high magnetic suceptibility anisotropy anymore [Tolman et al., 1995, Tjandra et al., 1996]. Initially application of RDCs to NMR structure determination centered on proteins [Bax and Tjandra, 1997, Tjandra and Bax, 1997, Tjandra et al., 1997], but subsequently the importance of RDCs for the structure determination of DNA was demonstrated extensively in theory [Vermeulen et al., 2000, Mauffret et al., 2002] and in practice [Tjandra et al., 2000, Zidek et al., 2001, MacDonald and Lu, 2002, Wu et al., 2004, Stefl et al., 2004].

Inducing just enough alignment for reliable measurement of RDCs, while retaining the high resolution and unambiguousness of the spectra is central to the success of RDC measurements. Tjandra and Bax [1997] showed that the degree of alignment can easily be adjusted by varying the concentration of the bicelles. In the following, diverse alignment media were developed in order to extend the applicability of the approach: bicelle-based alignment [Tjandra and Bax, 1997, Tjandra et al., 1997, Ottiger and Bax, 1999a, Barrientos et al., 2000, Al-Hashimi et al., 2000, Ruckert and Otting, 2000], filamentous phage [Hansen et al., 1998], stretched gels [Chou et al., 2001, Ma et al., 2008] or paramagnetic tagging [Wohnert et al., 2003]. The bacteriophage Pf1 proved to be particularly suited for aligning oligonucleotides since it is stable over a wide range of temperatures and Pf1-DNA interaction is minimized due to electrostatic repulsion between the two negatively charged macromolecules [Hansen et al., 1998, Prestegard et al., 2000]. The steric interaction of Pf1 with DNA is illustrated in Fig. 2.12 a.

RDCs (D_{ij}) are determined by measuring the difference of the dipolar coupling in the

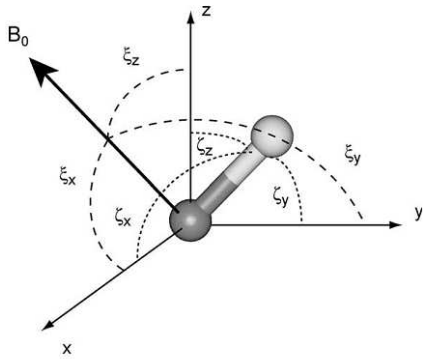


Fig. 2.13: *Orientation of the magnetic field, defined by angles ξ_x, ξ_y, ξ_z and the internuclear vector, described by angles $\zeta_x, \zeta_y, \zeta_z$, in the macromolecular frame. This picture is taken from Blackledge [2005].*

presence (${}^1J_{ij}^{ani}$) and absence of molecular alignment (${}^1J_{ij}^{iso}$), as is shown in Fig. 2.12 b.

$$ {}^1J_{ij}^{ani} = {}^1J_{ij}^{iso} + D_{ij} \tag{2.34} $$

The RDC term can be described by the following equation

$$ D_{ij} = -\frac{\gamma_i \, \gamma_j \, \mu_0 \, \hbar}{4 \, \pi^2} \left\langle \frac{3 \, cos^2 \alpha_{ij}(t) - 1}{2 \, r_{ij}^3(t)} \right\rangle \tag{2.35} $$

where α denotes the angle of the internuclear vector between atoms i and j with the applied magnetic field and $\langle\rangle$ designates the time and ensemble average. When no preferred alignment is induced, all values of α are sampled with equal probability over time and thus D_{ij} is averaged to zero. If residual order is induced by aligning the molecule, information on the orientation of the internuclear vector can be extracted. However, in order to have a fixed reference system, it is desirable to relate the orientation of the internuclear vector to the macromolecular frame rather than the magnetic field. To achieve this, two conditions must be met: 1) the distance between the atoms which give rise to D_{ij} does not change significantly over time (or else its distribution is known), such that r_{ij} can be substituted in eq. (2.35) with an effective distance r_{ij}^{eff} which is known and where averaging is already taken into account. 2) It is assumed that for macromolecules the time average of α can be expressed by two convoluted motions, the macromolecule tumbling with respect to the magnetic field vector (ξ_x, ξ_y, ξ_z) and the internuclear vector

moving inside the macromolecular frame $(\zeta_x, \zeta_y, \zeta_z)$ as illustrated in Fig. 2.13.

$$cos\,\alpha_{ij} = \begin{pmatrix} cos\,\xi_x \\ cos\,\xi_y \\ cos\,\xi_y \end{pmatrix} \begin{pmatrix} cos\,\zeta_x \\ cos\,\zeta_y \\ cos\,\zeta_y \end{pmatrix} = \sum_{k=}^{x,y,z} cos\,\xi_k\, cos\,\zeta_k \tag{2.36}$$

To a first approximation, the internuclear vector is considered to be fixed within the macromolecular frame and thus time-averaging has no effect on ζ. Introducing the alignment tensor $\mathbf{A}$ with dimensionless units

$$A_{kl} = \langle cos\,\xi_k\, cos\,\xi_l \rangle \tag{2.37}$$

eq. (2.35) can then be rewritten as

$$D_{ij} = -\frac{3\,\gamma_i\,\gamma_j\,\mu_0\,\hbar\,S_{flex}}{8\,\pi^2\,(r_{ij}^{eff})^3} \sum_{k,l=}^{x,y,z} \left(A_{kl}\, cos\,\zeta_k\, cos\,\zeta_l - \frac{1}{9}\,\delta_{kl} \right) \tag{2.38}$$

where S_{flex} represents an order parameter to account for local flexibility of the internuclear vector. Here the assumption is that the local motion does not influences the overall alignment of the macromolecule, which is reasonable in the absence of large amplitude fluctuations. In most cases, the local motion can be approximated with the diffusion-in-a-cone model, where the order parameter S_{flex} is related to the generalized order parameter S, which scales down the measured RDCs linearly [de Alba and Tjandra, 2002].

In the present framework the alignment tensor $\mathbf{A}$ has all elements non-zero. It is however desirable to find a specific molecular frame in which all off-diagonal elemtents of $\mathbf{A}$ are zero. Such a frame, called the principal axis system (PAS) can be found by a 3D Euler rotation of the current molecular frame with parameters α, β and γ [Blackledge, 2005]. Eq. (2.38) can then be rewritten in terms of the polar angles θ, ϕ (Fig. 2.14, left panel), which describe the orientation of the internuclear vector in the eigenframe of the

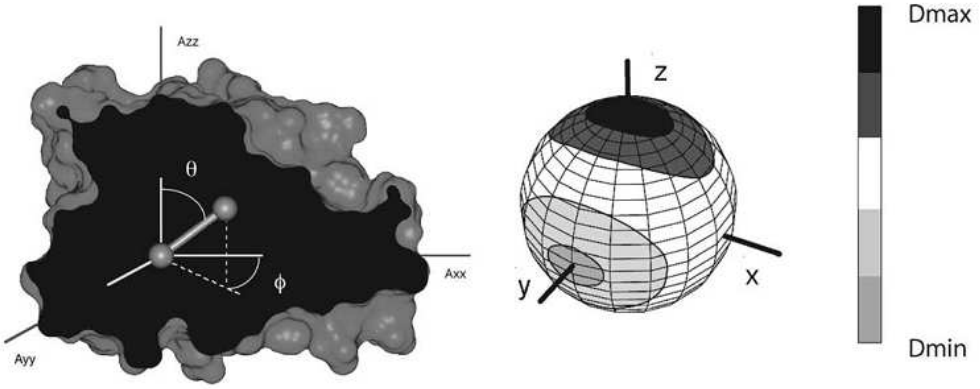


Fig. 2.14: *Orientation of the internuclear vector with polar coordinates θ and ϕ in the eigenframe of the alignment tensor with eigenvalues A_{xx}, A_{yy} and A_{zz} (left panel). The right panel illustrates the orientational degeneracy of RDCs. As can be clearly seen a large number of different orientations can be sampled when the RDC takes intermediate values. Only extreme RDC value define unique orientations of the internuclear vector. This picture is taken from Blackledge [2005].*

alignment tensor with eigenvalues $|A_{xx}| \leq |A_{yy}| \leq |A_{zz}|$ [Lipsitz and Tjandra, 2004]

$$D_{ij} = -\frac{\gamma_i \, \gamma_j \, \mu_0 \, \hbar \, S_{flex}}{4 \, \pi^2 \, (r_{ij}^{eff})^3} \left[A_a \, (3 \, cos^2\theta - 1) + A_r \, sin^2\theta \, cos \, 2\phi \right] \tag{2.39}$$

where A_a and A_r are the axial and rhombic components of the alignment tensor $\mathbf{A}$ [Prestegard et al., 2000], which are given by

$$A_a = \frac{1}{2} \, A_{zz} \quad and \quad A_r = \frac{1}{2}(A_{xx} - A_{yy}) \quad . \tag{2.40}$$

In consequence, the alignment tensor $\mathbf{A}$ is determined by 5 independent parameters: the three Euler angles needed for rotation of the reference frame (α, β, γ) and the two components of the alignment tensor $\mathbf{A}$ (A_a, A_r). The alignment tensor can then be unambiguously determined from a minimum set of 5 experimental RDCs by singular value decomposition [Losonczi et al., 1999].

With $\mathbf{A}$ at hand, the orientation of any internuclear vector with respect to the macromolecular frame can be calculated. Unfortunately, there exists a multitude of orientations of an internuclear vector that is consistent with a given intermediate RDCs value, as is illustrated in Fig. 2.14, right panel. Only extreme values correspond to unambiguous

orientations. The orientational degeneracy for intermediate RDC values clearly limits their value for structure determination. Thus it is desirable to remove this degeneracy by either measuring more RDCs per residue, or RDCs for the same internuclear vectors but with a different alignment tensor [de Alba and Tjandra, 2002, Blackledge, 2005].

2.3.4 Simulated Annealing calculations

The main idea behind Simulated Annealing (SA) calculations is coupling simple energy minimization to Molecular Dynamics. Thus the problem that molecules converge to a local minimum instead of the global can be circumvented. The Newtonian equation of motion

$$F_i(t) = -\frac{\partial V}{\partial r_i} = m_i\, a_i(t) \tag{2.41}$$

relates the acceleration $a_i(t)$ of each atom i at time t to the derivative of the potential energy (V) with respect to the atom position r_i. $F_i(t)$ represents the force which acts upon the atom i with mass m_i [Bloomfield et al., 2000]. The atoms are moved according to the force that is exerted upon them for a given time-step (typically between 1-5 fs). From the knowledge of the last and current atom positions and velocities, a new force is calculated which in turn acts on the atoms. This cycle is repeated until a convergence criterion (e.g. a minimum change in the gradient of the potential energy) is met. Initially, atom velocities are computed using a Gaussian or Maxwell distribution. The atom coordinates are derived from a starting structure. Since the initial coordinates and velocities determine all subsequent ones, it is important to start from a reasonable structure. This is usually achieved by starting from already known crystal or NMR structures.

Temperature-coupling of Molecular Dynamics is achieved by introducing an average kinetic energy which is computed via Boltzmann statistics

$$< E_{kin} >=< \frac{1}{2}\, m\, \nu^2 >= \frac{3}{2}\, k_b\, T \tag{2.42}$$

where k_b is the Boltzmann factor, m the atom mass and ν the atom velocity. The higher the temperature (T) is chosen, the higher the kinetic energy of the system. Thus at high temperatures kinetic barriers can be overcome, and the global minimum should be accessible.

The number of atoms in macromolecules such as DNA or proteins is on the order of thousands with three times as much cartesian coordinates to be calculated at each step. In order to make computation of macromolecules feasible, Molecular Dynamics calculati-

ons rely on the predefinition of atom types. For these atom types many parameters such as bond lengths, bond angles, dihedral angles, partial charges etc. are assumed to be fixed and are comprised in the force field. In the present work the program XPLOR-NIH [Schwieters et al., 2003] was used, which employs the CHARMM force field [Weiner et al., 1984, MacKerell et al., 2000]. The total potential energy V_{tot} consists of two components [Brünger, 1996]

$$V_{tot} = E_{emp} + E_{eff} \tag{2.43}$$

where the empirical (E_{emp}) and the effective energy term (E_{eff}) are given as [Brünger, 1996].

$$E_{emp} = E_{bond} + E_{angle} + E_{dihe} + E_{vdW} + E_{Coulomb} \tag{2.44a}$$

$$E_{eff} = E_{noe} + E_{rdc} + E_{plan} + E_{cdih} \tag{2.44b}$$

E_{bond}, E_{angle}, E_{dihe} and all energy terms of E_{eff} are calculated as the product of a scaling factor (the force constant) and the deviation of the observed value from the equilibrium one, e.g.

$$E_{bond} = k_{bond}\left(r_{ij}^{obs} - r_{ij}^{equ}\right) \tag{2.45}$$

The equilibrium values for E_{noe} and E_{rdc} are taken from experiment while these of E_{cdih} and E_{plan} are averages from the literature [Brünger, 1996]. The corresponding scaling factors are defined in the calculation input and thus can be used to increase the restraining power of selected energy terms. Equilibrium values and force constants of E_{bond}, E_{angle}, E_{dihe} constitute one part of the force field. E_{vdW} is given as

$$E_{vdW} = \sum_{i,j}\left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) \tag{2.46}$$

with $A_{ij} = 2\sqrt{\varepsilon_{ii}\,\varepsilon_{jj}}(\sigma_{ii} - \sigma_{jj})$ and $A_{ij} = 2\sqrt{\varepsilon_{ii}\,\varepsilon_{jj}}(\sigma_{ii} - \sigma_{jj})$. The atomic permitivities ($\varepsilon_{ii/jj}$) and van-der-Waals radii ($r_{ii/jj}$) set up another part of the force field. The partial

atomic charges (q_i, q_j) in the $E_{Coulomb}$-term constitute the last part of the force field.

$$E_{Coulomb} = \sum_{ij} \frac{q_i \, q_j}{\varepsilon_0 \, r_{ij}} \tag{2.47}$$

When explicit treatment of water is not feasible due to restrictions on the calculation time, the solvent screening effect can be approximated by introducing a distance dependent permittivity of free space $\varepsilon_0(r_{ij})$.

Ultimately, force field parameters are based on crystal structures and ab-initio calculations of small model molecules (bond lengths, bond angles and dihedral angles), infrared spectroscopy data (force constants) and empirical testing (where no experimental source is available) [Weiner et al., 1984]. When chemically modified nucleotides are to be incorporated into calculations, parameters for this nucleotide have to be added to the existing force field. In the absence of experimental data, these parameters are calculated using ab-initio methods.

3 Experimental details

3.1 2-Aminopurine

3.1.1 Sample preparation

NMR sample preparation DNA strands were obtained (from BIOTEZ, Berlin, and BIOSPRING, Frankfurt/M, Germany) already purified by reverse-phase high-pressure liquid chromatograpy (HPLC). After hybridization they were subjected to size exclusion chromatography (SEPHADEX G25) and ammonia treatment (lyophylization with $\approx 3\,\%$ NH_3-solution) to remove residual, low molecular weight impurities (mainly Et_3N-buffer from HPLC). Equivalent amounts of complementary single strands were hybridized by rapid heating to 90,°C and subsequent gradual cooling to room temperature at a rate of 1 °C per minute. NMR samples were prepared at 3 mM duplex concentration in D_2O (D_2O 99.98 %) and H_2O (H_2O:D_2O/90:10) at pH 6.5 in 10 mM Na_2HPO_4/NaH_2PO_4 and 150 mM NaCl solution.

RDC sample preparation Samples for measuring residual dipolar couplings (RDC) were prepared in D_2O as described above with the addition of 20 mg/ml Pf1 (obtained from ASLA BIOTECH, Riga) suspended in the same buffer. This necessitates rebuffering of Pf1 since it is obtained in a different and non-deuterated buffer. Rebuffering is achieved by ultracentrifuging 100 μl of Pf1 two times with 600 μl phosphate buffer (as described above) and subsequently two times with 600 μl deuterated phosphate buffer at 60000 rpm. Each ultracentrifuging step is performed at 4 °C for 2 h. Next the sediment is suspended in the DNA sample. The high viscosity of Pf1 complicates sample handling and thus the suspension has to be stirred until a viscose, clear, gel-like sample is obtained. After

transferring it into the NMR tube, bubbles have to be removed by slow centrifugation of the NMR tube (max. 500 rpm). The degree of orientation can be checked by measuring the quadrupolar deuterium splitting of the HOD signal [Lipsitz and Tjandra, 2004]. In case of degradation or non-complete suspension of Pf1 in the sample, the expected symmetric doublett with splitting of 10±5 Hz is asymmetric, extremely broadened or even non-observable.

3.1.2 Measurements

Duplex melting Melting of the 13mer2AP duplex was monitored by optical absorption at 260 nm and by the fluorescence quantum yield (due to the 2AP nucleobase) from 310 nm excitation [Evans et al., 1992]. For comparison, melting of the 13merRef duplex was measured by absorption only. The solutions had a total single-strand concentration of 23.7 mM and the optical path length was 1 cm. Temperature was varied between 25 and 85 °C; the standard error of transfer between absorption and emission temperatures was ± 0.011 °C. Following changes of typically 1 °C, equilibration was allowed for at least 15 minutes. The relative fluorescence yield of pure 2AP in buffered water was measured in the same manner for reference. Measurements were corrected for density changes.

Imino proton exchange Exchange rates of the imino protons were obtained from inversion recovery experiments at 298 K on a Bruker Avance 600 NMR spectrometer. For this purpose the standard 1D 1H-WATERGATE (water suppression by gradient tailored excitation) pulse program was modified to start with a selective inversion pulse. As discussed in section 2.2.2 the pulse shapes least sensitive to relaxation effects are rectangular and gaussian pulses. Dornberger et al. [1999] and Bhattacharya et al. [2002] use millisecond gaussian-shape pulses for inversion, but such long pulse durations again facilitate relaxation effects. Additionally, the imino protons are shifted upfield ($\approx$ 13 ppm) of the bulk of the DNA (1-9 ppm) and especially the HOD ($\approx$ 4.80 ppm) NMR signals and thus off-resonance excitation effects can be ruled out. In consequence, a selective rectangular inversion pulse of 327 μs duration centered on the imino proton region was employed in this work. After a variable delay with 21 settings ranging from τ=20 μs up to 2 ms, a 1D spectrum was recorded. The settings for the variable delay have been optimized to give accurate results for slowly as well as fast exchanging imino protons.

Samples were prepared in H_2O with the buffer described earlier. In order to cancel the effect of intrinsic catalysis (cf. section 2.2.1), the spin-lattice relaxation time T_1^{int} of all imino protons was measured prior to titration with base catalyst. Subsequently, each duplex was titrated with 1 M TRIS buffer as base catalyst for proton exchange. Test runs

with ammonia as base catalyst gave inferior results, since the latter is highly volatile. This makes high-concentration salt solutions of ammonia, which are necessary in order to keep the pH stable upon addition of the catalyst, inaccessible. Another set of test runs, where pure ammonia and HCl (for pH compensation) were added separately to the NMR sample, resulted in temporary but extreme pH changes (pH values ranging from 1 to 11). These changes led to gradual degradation of the DNA sample. Furthermore, the error in concentration introduced by evaporation of ammonia is intolerable when high precision results are to be obtained. In order to keep the pH as stable as possible, TRIS-hydrochloride at pH 7.4 was used. At every titration point the pH was measured in order to determine the amount of free base catalyst precisely (cf. eq. (2.15 in section 2.2.1). All pH values centered around 7.1. To estimate the error in T_1-determination, three sets of measurements were carried out at each titration point, resulting in 693 1D spectra (21 delay settings, 11 titration points).

Each spectrum was fitted (with the MATHEMATICA program environment) to give the integrals of the imino proton peaks. Complete spectral fitting became necessary since standard region integration as implemented in TOPSPIN is sensitive to peak overlap. Due to continued broadening of the imino proton signals upon base catalyst addition, integrals obtained by the latter method were inconsistent and thus gave rise to large errors (up to 100 %) in T_1-values. In contrast, complete spectral fitting in MATHEMATICA yielded T_1-values with errors of typically 0.5-5 %.

Structural NMR NMR measurements for structure calculation were carried out on a BRUKER AVANCE 600 NMR spectrometer. The optimum temperature of 298 K was determined by monitoring the imino proton signal intensity (cf. Fig. 3.1). For each duplex, NOESY-, DQF-COSY- (double quantum filtered correlated spectroscopy), TOCSY- (total correlation spectroscopy) and HMQC-spectra (heteronuclear multiple quantum coherence spectroscopy) in D_2O, a WATERGATE-NOESY-spectrum in H_2O and an HMQC-spectrum in D_2O/Pf1 were recorded. The quadrupolar splitting of the ^2H NMR signal after addition of Pf1-phage was 15.72 Hz and 13.65 Hz for 13merRef and 13mer2AP, re-

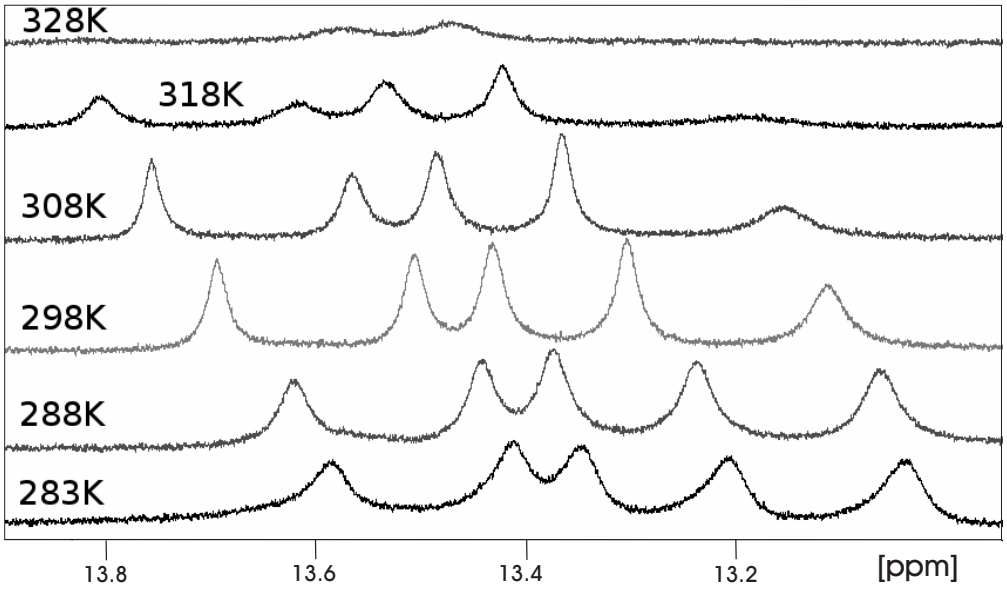


Fig. 3.1: *Imino proton signal intensities for the temperature range 283-328 K. in 13mer2AP.*

spectively. Upon addition of Pf1, the sample became very viscose and standard shimming procedures failed to produce reasonable linewidths. Two workarounds were investigated. One was to optimize the shim for a BRUKER standard sample and use these shim settings on the DNA/Pf1 sample. The second workaround involved opimization of the lineshape of the HOD signal. This procedure demands that after every change of the shim settings, a 1D spectrum of the sample had to be aquired. Although the latter procedure is more time-consuming than the other one, it yielded supreme results and thus was employed.

For DQF-COSY- (TOCSY-) spectra, 16 (32) transients were acquired, with 2048×256 points in F2 and F1 dimensions. For NOESY-spectra in both solvents, 16 transients were acquired with 4096×2048 points at a mixing time of 150 ms. For the HMQC-spectra with and without Pf1, 192 transients were acquired with 8192×512 points. The high number of data points was necessary to obtain sufficient resolution in the proton dimension in order to determine the RDC-values with a precision below 1 Hz. The optimal d2-delay, where both the non-aromatic and aromatic region are equally enhanced, was determined to be 2.5 ms. All spectra were processed with the BRUKER TOPSPIN-software, and signals were assigned with the help of CARA [Keller, 2004].

3.1.3 Restraint generation

Force field parametrization Density functional theory (DFT) calculations of the 2AP-nucleotide were performed with GAUSSIAN03 employing a triple zeta valence plus polarisation (TZVP) basis set. Due to the structural proximity of 2AP and A, force field parameter and topology entries of the latter were adopted and changed where necessary. Special emphasis was placed on the charge distribution since this was shown to be the main difference between A and 2AP [Broo, 1998, Mishra et al., 2000, Jean and Krueger, 2006]. For this purpose several methods for obtaining point charges were compared. The fastest method is the Mulliken population analysis, which, however, gives unrealistic results [Breneman and Wiberg, 1990]. This is due to the equal distribution of overlap populations between the two involved atoms. Hence another approach has been developed, which tries to derive atomic charges via fitting of point charges to the Molecular Electrostatic Potential (MEP) [Connolly, 1983, Singh and Kollman, 1984]. Crucial to the success of this technique is the algorithm which is used for fitting. Three different algorithms were tested; the CHELP- [Chirlian and Francl, 1987], the CHELPG- [Breneman and Wiberg, 1990] and the MK-algorithm [Besler et al., 1990]. Out of these three the MK- and CHELPG-algorithms gave consistent and similar results. Partial charges derived with the CHELPG-algorithm were employed in the force field. Although the sugar moiety was simulated as well, only the partial charges for the 2AP residue were integrated into the force field. Due to the similarity of A and 2AP the influence of the latter on the partial charges of the sugar can be considered negligible (as the same values for partial charges of sugar atoms are also used for G,C and T residues). Thus the partial charge values for the sugar were taken as already defined for the native residues in the force field. To retain the neutrality of the 2AP residue the partial charge at the N7 atom of 2AP was increased from -0.61 to -0.45 (cf. section 3.1.3).

Distance restraints The assigned NOE cross-peaks were converted to distance restraints by referencing their integrals to the integrals of known distances employing the Isolated Spin Pair Approximation. The NOE cross-peaks were integrated with the pro-

gram CARA [Keller, 2004] using the sum-over-rectangle method. As reference distances Me-H6 T (3.1 Å) for all NOE cross-peaks involving methyl protons, H42-H5 C (2.4 Å) for all NOE cross-peaks involving exchangeable protons and H5-H6 C (2.5 Å) for the remaining NOE cross-peaks were used (bond lengths adapted from the force field parameters). For the purpose of exporting the integral values obtained by CARA [Keller, 2004] to an XPLOR-NIH [Schwieters et al., 2003] restraints file, a LUA script was written (see section .4). This script classifies the intregrated peaks according to the overlap with other peaks and scales their volume integrals accordingly. Additionally, uncertainties for the NOE restraints are automatically calculated from the standard deviation of the reference peaks' volume integrals. The estimated uncertainty is then increased according to the classification of each peak. Furthermore, this classification is printed into a separate file, which can be used to assess whether or not peak overlap might prevent a reliable estimation of the peak volume.

Residual Dipolar Coupling restraints The experimentally determined RDCs (see tables 3.1 and 3.2) of C-H bond vectors can be input into the structure calculations with the measurement precision as error bounds. From the experimentally determined RDC-values the orientation of the corresponding internuclear vector is determined via eq. (2.39) in section 2.3.3. All constants in the latter equation are comprised within the factor D_a

$$D_a = -\frac{\gamma_i \, \gamma_j \, \mu_0 \, \hbar \, S_{flex}}{4 \, \pi^2 \, (r_{ij}^{eff})^3} \tag{3.1}$$

for which only one value is used throughout the calculation. This necessitates scaling of different sets of RDCs, for example when C-H as well as C-C RDCs have been measured. Scaling is achieved by introducing prefactors to the D_a-factor, which are defined as the ratio of the two D_a-factors involved. For example, when C-C RDCs are to be scaled to a set of C-H RDCs, the corresponding prefactor would be calculated as:

$$D_a^{pre}(CC) = \frac{D_a(CH)}{D_a(CC)} = \frac{\gamma_H}{\gamma_C} \left(\frac{r_{CC}}{r_{CH}}\right)^3 = \frac{42.576}{10.705} \left(\frac{1.496}{1.090}\right)^3 \approx 10.28 \quad . \tag{3.2}$$

where $\frac{\gamma_H}{\gamma_C}$ is the change in gyromagnetic ratio when working with C-H or C-C RDCs and r_{CC} and r_{CH} are the C-C and C-H bond lengths between the atoms defining the internuclear vector. Although in this work only C-H RDCs were measured, scaling issues become important for the implementation of the experimentally determined C-H RDCs of the T methyl groups.

Due to the fast rotation of the latter, only an averaged value for all three C-H bond vectors can be measured. This fast rotation scales the corresponding RDC values by

$$P_2(cos\beta) = \frac{3}{2}\, cos^2\beta - \frac{1}{2} \tag{3.3}$$

where β is the C5-C7-H7* angle. The latter is usually assumed to be the ideal tetrahedral angle of 109.5 °, but was experimentally determined for methyl groups to be 110.9 ° by Ottiger and Bax [1999b]. Although small, the deviation from the ideal angle has a strong impact on methyl RDC scaling since $P_2(\cos\beta)$ is a steep function β. Thus with $P_2(\cos 109.5)$=-0.3329 and $P_2(\cos 110.9)$=-0.3089, conversion factors between C5-C7 and C7-H7* methyl RDCs can be calculated as -3.42 and -3.17, respectively, according to

$$\frac{D_{CH\_Me}}{D_{CC\_Me}} = P_2(cos\beta)\, D_a^{pre}(CC) \quad . \tag{3.4}$$

The value of -3.17 was determined by measuring the correlation of experimentally determined C-H and C-C methyl RDCs [Ottiger and Bax, 1999b].

In order to implement the methyl C-H RDCs into the structure calculations, they have to be associated with a unique internuclear vector. Thus it is necessary to convert the C7-H7* RDCs into the corresponding C5-C7 RDCs. Since the latter must now be input as C-C RDCs, a seperate restraints file has to be used. There are in principle two ways how this implementation can be achieved in Xplor-NIH v2.20 [Schwieters et al., 2003]. One way is to convert all experimentally determined methyl C-H RDC values by hand to the corresponding C-C values with the factor 1 / -3.17 = -0.3155. This is done prior to implementing them into the calculation. In that case, the prefactor D_a^{pre}(CC) can be used to scale the C-C RDC input file to the C-H RDC input. According to the manual,

this scaling is handled automatically by the *scale_toCH* routine of the program XPLOR-NIH v2.20 [Schwieters et al., 2003]. Due to a bug however, the automatic scaling is only effective when the methyl C-C RDCs are input as type "CACO". The other way to input C-H methyl RDCs into structure calculations will be implemented in the new version of XPLOR-NIH (v2.24) but was made available to the author in advance (personal communication of Dr. Charles Schwieters). In that new version, the methyl RDCs are input as a separate restraints file too, but their values do not have to be scaled by hand. They are automatically recognized by the program as methyl RDCs and scaled to the C-H RDC input by

$$D_{Me}^{pre} = -3.17\, D_a^{pre}(CC) = 32.59 \quad . \tag{3.5}$$

However, scaling of the D_a-factor and scaling the RDC values is not equivalent, since the energy for the RDC potential term is given by [Brünger, 1996]

$$E_{RDC} = k_{RDC}\,(D_{calc} - D_{obs})^2 \tag{3.6}$$

where k_{RDC} is the scale factor for the RDC energy term. The latter must be modified for different sets of RDCs according to their respective errors with a weighting factor ω_{ij}. When using the prefactor D_{Me}^{pre} for implementation of methyl RDCs, the energy of the C-C methyl RDCs has to be scaled by

$$\omega_{CC} = \frac{1}{(-3.17)^2}\,\omega_{CH} = 0.0995\,\omega_{CH} \tag{3.7}$$

due to dependence of the energy on the square of the difference between calculated (D_{calc}) and observed (D_{obs}) RDC.

Tabelle 3.1: *Experimentally determined RDCs for 13merRef. The RDCs were measured with a precision of ±0.6 Hz.*

Res	Vector	$J_{(CH)}$ (Hz)	$J_{(CH)}$(aligned) (Hz)	RDC (Hz)
A6	C2-H2	201.6	225.6	24.0
A7	C2-H2	203.4	224.4	21.0
A8	C2-H2	202.2	223.8	21.6
A16	C2-H2	202.8	214.8	12.0
A24	C2-H2	201.6	224.4	22.8
C9	C5-H5	171.6	190.2	18.6
C17	C5-H5	170.4	183.6	13.2
T3	C7-H7	126.6	122.4	-4.2
T11	C7-H7	126.0	118.8	-7.2
T19	C7-H7	123.0	112.8	-10.2
T20	C7-H7	127.2	118.2	-9.0
T21	C7-H7	126.6	118.2	-8.4
T3	C1'-H1'	155.4	167.4	12.0
C9	C1'-H1'	162.0	169.2	7.2
C17	C1'-H1'	162.6	166.8	4.2
T20	C1'-H1'	163.8	178.2	14.4
T21	C1'-H1'	153.6	174.0	20.4
T3	C6-H6	177.0	195.0	18.0
C5	C6-H6	180.0	201.0	21.0
T20	C6-H6	177.0	192.6	15.6
T21	C6-H6	175.8	190.8	15.0
C23	C6-H6	175.8	201.0	25.2
A7	C8-H8	213.0	235.8	22.8
A16	C8-H8	213.0	230.4	17.4

Tabelle 3.2: *Experimentally determined RDCs for 13mer2AP. The RDCs were measured with a precision of ±0.6 Hz.*

Res	Vector	$J_{(CH)}$ (Hz)	$J_{(CH)}$(aligned) (Hz)	RDC (Hz)
A6	C2-H2	202.2	222.0	19.8
2AP7	C6-H6	179.4	200.4	21.0
A8	C2-H2	202.8	218.4	15.6
A16	C2-H2	201.6	213.6	12.0
A24	C2-H2	201.6	219.0	17.4
C5	C5-H5	170.4	184.2	13.8
C9	C5-H5	171.0	189.6	18.6
C17	C5-H5	170.4	183.0	12.6
T3	C7-H7	127.2	121.2	-6.0
T11	C7-H7	127.2	121.2	-6.0
T19	C7-H7	126.6	118.2	-8.4
T20	C7-H7	127.2	120.6	-6.6
T21	C7-H7	126.6	119.4	-7.2
T3	C1'-H1'	160.8	172.2	11.4
2AP7	C1'-H1'	156.6	170.4	13.8
C9	C1'-H1'	160.8	168.6	7.8
C17	C1'-H1'	162.6	166.2	3.6
T21	C1'-H1'	161.4	182.4	21.0
T3	C6-H6	175.2	193.8	18.6
C5	C6-H6	175.2	195.6	20.4
T20	C6-H6	176.4	190.2	13.8
T21	C6-H6	176.4	192.0	15.6
C23	C6-H6	174.0	199.2	25.2
2AP7	C8-H8	214.2	234.6	20.4
A16	C8-H8	213.6	229.8	16.2

3.1.4 Structure calculation

Calculation input Structure calculations were performed with XPLOR-NIH v2.20 [Schwieters et al., 2003]. A total of 340 (333) NOE distance restraints and 24 (25) Residual Dipolar Couplings were used for 13merRef (13mer2AP). The experimental data were supplemented with 144 backbone dihedral restraints, 78 hydrogen bond distance restraints and 28 planarity restraints (see Table 3.3).

Initial MD-calculations were performed with dihedral restraints allowing both A-form and B-form conformations (with error bars of $\pm 50\,^{\circ}$). B-form conformation was experimentally confirmed by ^{3}J coupling constants for H1'-H2' derived by P.E.COSY (primitive exclusive correlated spectroscopy) and NOESY-cross-peak intensities characteristic of B-DNA. Consequently, regular dihedral values from the literature [Roberts, 1993] were included in the calculations.

Tabelle 3.3: *Overview of structural statistics for 13merRef and 13mer2AP calculations*

	13merRef	**13mer2AP**
NOE restraints		
- total	340	333
- interresidue	196	188
- intraresidue	144	145
- 2AP to DNA	-	9
RDC restraints	24	25
Dihedral angle restraints	144	144
H-bond restraints	78	78
Base pair planarity restr.	28	28
NOE viol. (> 0.5 Å)	0	0
RDC viol. (> 0.4 Hz)	0	0
Dihe viol. ($> 5\,^{\circ}$)	0	0
RMSD to ave. struct. in Å	0.30	0.33

The structures were calculated in two steps. First, a reasonable starting structure with well defined local conformation was computed. To ensure that no bias is introduced towards local energy minima, the calculation started from an elongated and equilibrated structure. The resulting structure, which is mainly defined by NOE restraint data, was used as input for Simulated Annealing calculations including RDC data. The need for locally well defined starting structures in order to calculate reasonable structures which

satisfy NOE as well as RDC data is documented in the literature [Vermeulen et al., 2000, Mauffret et al., 2002].

Simulated Annealing protocol The two complete MD protocols are given in the Appendix, section .2. Only the protocols for the 13mer2AP calculation is given, since the other calculations were carried out with the same protocol (with the exception of the input file names). The input scripts are based on the example files of the XPLOR-NIH package (refine_full.py and sa.inp) but were substantially modified in the course of this work.

The MD protocol with only NOE restraints as experimental input consisted of an initial minimization (50 steps) followed by 48 ps of high-temperature cartesian coordinate dynamics at 3000 K, subsequent gradual cooling to 25 K in 120 steps of 0.05 ps length and a final minimization (3000 steps).

The MD protocol including the experimental RDC restraints consisted of an initial cartesian coordinate minimization (1000 steps) followed by 50 ps of high-temperature cartesian coordinate torsion angle dynamics at 20000 K, subsequent gradual cooling to 25 K in 154 steps of 0.5 ps length (34 steps to cool down to 3000 K, followed by 120 steps to reach the end temperature) and a final minimization (3000 steps). The alignment tensor values were allowed to float during the calculations, as implemented in XPLOR-NIH (v2.20) [Schwieters et al., 2003].

For each run an ensemble of 100 structures was computed. The 10 minimum energy structures without violation of restraints were chosen to compute an averaged structure which was energy-minimized to yield the final structure. The root-mean-squares-deviation (RMSD) of the 10 minimum energy structures to the average, minimized structure is a measure for the precision of the calculation.

Structure validation To check the accuracy of the structures, NOESY-spectra were back-calculated from the average structures with the Full Matrix Relaxation Approach implemented in XPLOR-NIH (v2.20) [Schwieters et al., 2003]. The back-calculated spectra were visualized with the program GIFA [Pons et al., 1996] and overlayed with the

experimental ones. Furthermore, RDCs were predicted from the average structure using the program PALES [Zweckstetter and Bax, 2000] and compared to the experimental ones. Finally, helical parameters were calculated with the help of the program 3DNA [Lu and Olson, 2003]. These were checked for consistency with values for regular B-DNA helices.

A number of convenience scripts were written to automate data conversion between PALES [Zweckstetter and Bax, 2000] and XPLOR-NIH (v2.20) [Schwieters et al., 2003], the latter program and GIFA [Pons et al., 1996] and for quick access to energies or number of restraints in structure calculations output files. These utility scripts are comprised in the Appendix in section .5.

3.2 2-Hydroxy-7-nitrofluorene

Since many experimental details for HNF are equivalent to the ones for 2AP, the description for the latter is referenced where appropiate in order to avoid redundance.

3.2.1 Sample preparation

2-Hydroxy-7-nitro-fluorene was synthesized (Matthias Pfaffe) in four steps. The 2'-deoxyriboside dRi-HNF was prepared by reaction with 1'α-Chloro-3',5'-di-O-toluoyl-2'-deoxy-D-ribose in the presence of activated molecular sieve. Quantification of the H1'-H2'/H2" and H3'-H2'/H2" NOE cross-peaks in the NOESY-spectra of the main product revealed that the α-glycoside was formed predominantly. After purification of the latter by column chromatography, the corresponding phosphor-amidite was reached by standard methods. Fixed-phase synthesis of the labelled DNA strand (BIOTEZ) required a fourfold increase over the normal reaction time for coupling dRi-HNF. Further sample preparation is equivalent to the one described in section 3.1.1.

3.2.2 Measurements

Duplex melting Melting of the 13merHNF duplex was monitored by optical absorption at 260 nm and by the red-shift of the weak absorption band of HNF at 380 nm. The solution had a total single-strand concentration of 23.5 mM and the optical path length was 1 cm. The following procedures are equivalent to the ones described in section 3.1.2.

Structural NMR NMR measurements for structure calculation were carried out on a Bruker Avance 600 NMR spectrometer at 298 K. For each duplex, NOESY-, DQF-COSY-, TOCSY- and HMQC-spectra in D_2O, a WATERGATE-NOESY-spectrum in H_2O and an HMQC-spectrum in D_2O/Pf1 were recorded. The quadrupolar splitting of the ^2H NMR signal after addition of Pf1-phage was 10.55 Hz. The following procedures and settings are equivalent to the ones described in section 3.1.2.

3.2.3 Restraint generation

Distance restraints, RDC restraints and force field parameters were generated as described in section 3.1.3. The experimental RDC values that were used for restraint generation are given in Table 3.4. In the case of HNF however, no topology information for a structurally similar compound was available. Thus the corresponding parameter and topology input files had to be generated from the structure calculated by DFT methods as described in section 3.1.3. The latter can be achieved utilizing the program XPLO2D [Kleywegt and Jones, 1998].

The influence of the HNF on the partial charges of the sugar residue was considered to be non-negligible for C1' and the directly bonded atoms due to the strong structural and electronic differences between native nucleobases and HNF. Thus, in contrast to 2AP, for the atoms C1', C4', H4', H1", and O4' of HNF the partial charges derived from the DFT-calculations were used. The charge at O4' was increased by +0.13 in order to retain neutrality of the residue (cf. section 3.1.3).

Tabelle 3.4: *Experimentally determined RDCs for 13merHNF. The RDCs were measured with a precision of ±0.6 Hz.*

Res	Vector	$J_{(CH)}$ (Hz)	$J_{(CH)}$(aligned) (Hz)	RDC (Hz)
A6	C2-H2	202,2	228,0	25,8
A8	C2-H2	201,0	223,8	22,8
A16	C2-H2	201,6	220,8	19,2
A24	C2-H2	201,0	229,8	28,8
T3	C7-H7	124,8	120,0	-1,6
T11	C7-H7	125,4	117,0	-2,8
HNF7	C1'-H1"	169,2	182,4	13,2
C9	C1'-H1'	163,2	172,2	9,0
C17	C1'-H1'	161,4	166,2	4,8
T3	C6-H6	176,4	198,0	21,6
A6	C8-H8	213,6	244,2	30,6
A8	C8-H8	215,4	238,8	23,4
A16	C8-H8	213,6	234,0	20,4
A24	C8-H8	214,8	240,6	25,8
HNF7	C6-H6	157,8	189,0	31,2
HNF7	C8-H8	162,0	191,4	29,4
ABA20	C1'-H1'	147,0	147,0	0,0
ABA20	C1'-H1"	145,8	148,2	2,4

3.2.4 Structure calculation

The Simulated Annealing protocol and Structure validation were carried out as described in section 3.1.4.

Calculation input Structure calculations were performed with XPLOR-NIH v2.20 [Schwieters et al., 2003]. As the HNF was found to exist in two different orientation with in the duplex, two calculation runs were performed with the HNF methylene group pointing towards the major or minor groove for face-up and face-down orientations, respectively. A total of 401 (403) NOE distance restraints and 19 Residual Dipolar Couplings were used for the calculation of the face-up (face-down) orientation. The experimental data were supplemented with 124 backbone dihedral restraints, 72 hydrogen bond distance restraints and 24 planarity restraints (see Table 3.5).

Initial MD-calculations were performed with dihedral restraints, allowing both A-form and B-form conformations (with error bars of $\pm 50\,^{\circ}$). B-form conformation was experimentally confirmed by NOESY-cross-peaks intensities characteristic of B-DNA. Consequently, regular dihedral values from the literature [Roberts, 1993] were included in the calculations for all but the HNF residue.

Tabelle 3.5: *Overview of structural statistics for 13merHNF calculations*

	face-up	**face-down**
NOE restraints		
- total	401	403
- interresidue	225	226
- intraresidue	176	177
- 2AP to DNA	33	31
RDC restraints	19	19
Dihedral angle restraints	124	124
H-bond restraints	72	72
Base pair planarity restr.	24	24
NOE viol. ($> 0.5\,$Å)	0	0
RDC viol. ($> 0.4\,$Hz)	0	0
Dihe viol. ($> 5\,^{\circ}$)	0	0
RMSD to ave. struct. in Å	0.63	0.46

4 Results and discussion

4.1 2-Aminopurine

4.1.1 Introduction

2AP is known since the 1950s for its role in the mutagenic transition of A:T to G:C base pairs [Freese, 1959, Rogan and Bessman, 1970, Ronen, 1979]. As a structural isomer of A it can form stable WC-type base pairs with T (see Fig. 4.1) [Ronen, 1979, Sowers et al., 1986]. Base pairs with C, A or G are much weaker [Sowers et al., 1986, Fazakerley et al., 1987, Hochstrasser et al., 1994, Law et al., 1996]. Thus 2AP is incorporated instead of A by DNA polymerase opposite to T [Freese, 1959], though at a lower frequency than A [Rogan and Bessman, 1970, Watanabe and Goodman, 1982]. The mutagenic transition of A:T to G:C occurs during replication of 2AP:T base pairs [Bernstein et al., 1976, Goodman et al., 1977]. By adjusting the type of polymerase [Grossberger and Clough, 1981] or the concentration of nuclease [Clayton et al., 1979] the mutagenic rate can be tuned. The influence of nucleotide sequence on the mutagenic rate has no discernable logical pattern [Pless and Bessman, 1983]. Another way to stimulate the mutagenic transition is to increase the dCTP- or decrease the TTP-level

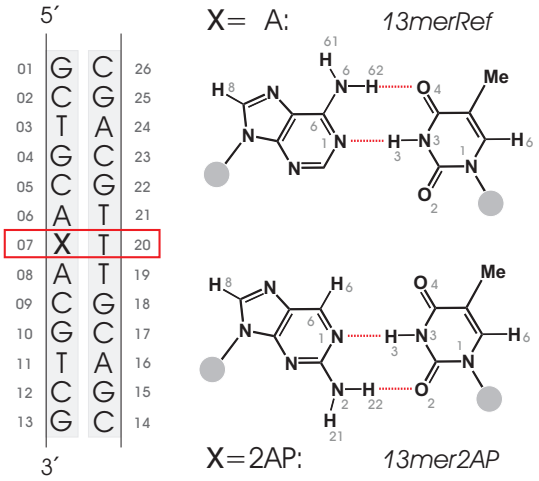


Fig. 4.1: *DNA duplex sequence with chemical structure of the 2AP-T and A-T base pairs.*

in the cell [Caras et al., 1982]. In 1981 Watanabe et al. [Watanabe and Goodman, 1981] demonstrated that 2AP:C mispairs form at 320-fold higher frequency than A-C mispairs. They concluded that 2AP:C mispairing is central to the mutagenicity of 2AP.

The stabilization of the 2AP:C base pair in solution therefore had to be investigated. Four models have been proposed for the geometry of this mispair. Ronen [1979] suggested a WC geometry with one hydrogen bond, while Goodman and Ratliff [1983] presented evidence for two hydrogen bonds involving one of the bases in the rare tautomeric imino form. Sowers et al. put forth the other two pairing schemes. Based on NMR imino proton data and pH-titration experiments, they proposed WC geometry through protonation of the mispair at neutral pH [Sowers et al., 1986]. A ^{15}N-enriched NMR-study later suggested a wobble geometry at high pH [Sowers et al., 1989], corroborated by fluorescence anisotropy measurements which showed pH-dependent conformational changes in the geometry of the 2AP:C mispair [Guest et al., 1991]. ^{15}N-enriched NMR-studies by Fagan et al. [1996] and Sowers et al. [2000] reproduced this pH-dependence and could finally demonstrate that the wobble geometry is predominant at neutral pH while protonation occurs sequence-dependently at lower pH. This conclusion is supported by a theoretical study [Sherer and Cramer, 2001].

The fluorescence properties of 2AP differ markedly from those of the natural nucleobases [Longworth et al., 1966, Callis, 1979, Serrano-Andres et al., 2006]. This was recognized originally by Ward et al. [1969] who observed a decrease in quantum yield of fluorescence by two orders of magnitude upon stacking of 2AP in a DNA helix. The effect was first exploited to determine thermodynamic parameters of stacking associations by Bierzynski et al. [Bierzynski et al., 1977b,a, Gajewska et al., 1982].

Fluorescence quenching of 2AP can also be used to study structural transitions in biological systems [Ward et al., 1969]. At the beginning such applications were thwarted by the lack of site-specific incorporation techniques. After chemical synthesis of 2AP-containing DNA-duplexes was realized [Eritja et al., 1986, McLaughlin et al., 1988], fluorescence quenching of 2AP was employed to study structural transitions in DNA [Lycksell et al., 1987, Patel et al., 1992]. In 1993 Bloom et al. [Bloom et al., 1993, 1994] were the

first to use 2AP fluorescence quenching to follow the kinetics of polymerase-catalyzed 2AP-insertion on a millisecond timescale. But on the whole, fluorescence quenching of a 2AP nucleobase surrogate is used to detect base stacking-unstacking transitions.

The local dynamics in the vicinity of 2AP, and its variation with sequence, can be studied by monitoring the fluorescence anisotropy decay [Nordlund et al., 1989]. This technique was used by Guest et al. [1991] to characterize the dynamic behaviour of mismatched base pairs. Hochstrasser et al. [1994] combined both techniques - fluorescence quenching and anisotropy decay - to study DNA double strand melting upon binding to the Klenow fragment. Xu et al. [1994] utilized 2AP fluorescence to show that DNA duplex melting proceeds via a highly flexible, yet B-DNA type transition state.

Numerous studies - facilitated by improved synthesis [Fujimoto et al., 1996] and subsequent commercial availability of 2AP-containing oligonucleotides - exploit 2AP fluorescence to investigate various problems in structural biology and biophysics: methyltransferase-induced base flipping [Allan and Reich, 1996, Allan et al., 1998, Holz et al., 1998, Allan et al., 1999, Stivers et al., 1999, Gowher and Jeltsch, 2000, Reddy and Rao, 2000, Bernards et al., 2002, Daujotyte et al., 2004, Su et al., 2004, Neely et al., 2005, Lenz et al., 2007], conformational changes and enzymatic cleavage of the hammerhead ribozyme [Menger et al., 1996, 2000, Kirk et al., 2001], promoter binding and clearance of T7 RNA polymerase [Jia et al., 1996, Ujvari and Martin, 1996, Bandwar and Patel, 2001, Liu and Martin, 2002], binding and strand separation of primer-template DNA by T4 DNA polymerase [Marquez and Reha-Krantz, 1996, Beechem et al., 1998, Fidalgo da Silva et al., 2002, Mandal et al., 2002, Hariharan and Reha-Krantz, 2005, Hariharan et al., 2006, Tleugabulova and Reha-Krantz, 2007], and charge transfer mechanisms together with polar solvation in DNA [Kelley and Barton, 1999, Wan et al., 2000, Fiebig et al., 2002, Jean and Hall, 2002, O'Neill and Barton, 2002a,b, Jean and Hall, 2004, O'Neill and Barton, 2004, Hardman and Thompson, 2006, Jean and Krueger, 2006, Hardman and Thompson, 2007]. All of these studies utilize 2AP fluorescence.

A new possibility to study structural transitions in biomolecules has recently been devised which makes use of unique properties of 2AP. Johnson et al. [2004] demonstrated

that a low-energy Circular Dichroism band is observed for 2AP dinucleotides incorporated into DNA or RNA double strands. They used this method to monitor structural changes in RNA hairpin loops [Johnson et al., 2005b] or breathing fluctuations at replication forks [Johnson et al., 2005a]. This new technique and the high number of 2AP-related publications show the significance of 2AP for the study of biological macromolecules.

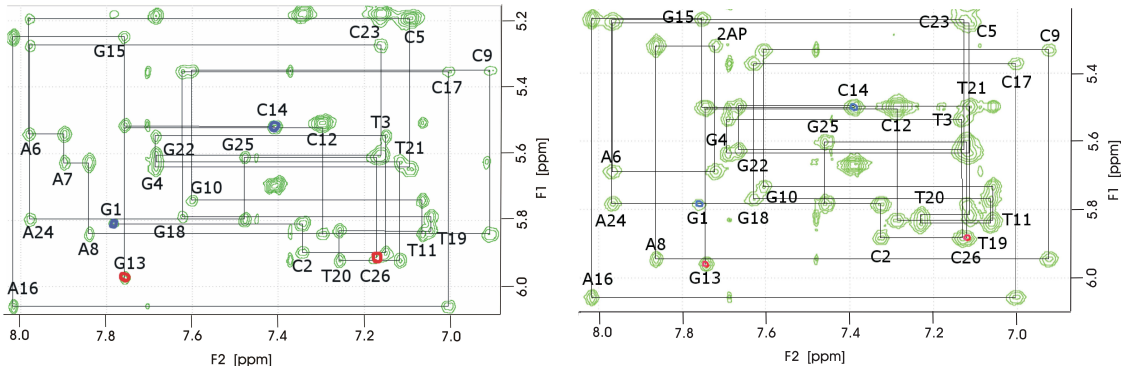
Structure perturbation upon incorporation of 2AP into a DNA-helix was investigated in detail only once, by Nordlund et al. [1989]. A palindromic sequence containing two 2AP:T base pairs was investigated by NMR spectroscopy, fluorescence decay and Molecular Dynamics simulations. Base pairing is observed in the duplex, but the melting temperature of the duplex was considerably lower than in the reference with WC A:T base pairs. The missing of NOE-crosspeaks to the 5'-side of the 2AP-substitution was interpreted as a local disturbance of the overall B-type helix [Nordlund et al., 1989]. Studies on minor groove binding drugs support this conclusion. They indicate that 2AP incorporation into A-T tracts reduces the binding affinities of these drugs to the level of G-C tracts [Loontiens et al., 1991, Patel et al., 1992]. Minor groove distortions by 2AP have also been proposed by a theoretical study [Lankas et al., 2002].

Dynamical effects due to incorporation of 2AP were examined even more scarcely. Nordlund et al. [1989] reported efficient recognition and cleavage of 2AP-containing duplexes by EcoRI, and concluded that dynamics are not perturbed. On the contrary, Lycksell et al. [1987] observed a 1 ms lifetime for the 2AP:T base pair, lower than for the corresponding A:T base pair, but state that the accuracy is correct probably only within a factor of 2. A destabilizing effect on the neighbouring pairs was not observed.

When structural transitions in biological systems are studied with a molecular probe, the assumption is that the modified system behaves like the natural one. Consequently the introduction of the probe must leave the structure unchanged. Disturbance of the original helix when 2AP occupies an A site would limit or even prevent the use of 2AP-fluorescence in some studies of biological systems. Therefore the above-mentioned hypothesis of a local disturbance has to be tested.

For this purpose a detailed analysis of the solution structure of a DNA-duplex in which

an A is substituted by 2AP is presented. The only change introduced into the helix is the difference in the position of the amino group of A and 2AP (cf. Fig. 4.1). In contrast to previous studies [Lycksell et al., 1987, Nordlund et al., 1989] the nonpalindromic nature of the sequence allows to have a single perturbation site. Thus any change in structure or base pair dynamics can be directly attributed to the 2AP incorporation. The central base pair was modified in order to detect possible long-range effects (up to three base pairs) of 2AP incorporation, which otherwise might be rendered ambiguous due to base pair fraying effects [Nonin et al., 1995]. A symmetric 13 base pair sequence was chosen to dismiss the possibility of mispairing, loop formation and fraying effects.



(a) *13merRef* **(b)** *13mer2AP*

Fig. 4.2: *Sequential assignment in the sugar H1' base H6/H8 region of the NOESY-spectrum in D_2O for 13merRef (a) and 13mer2AP (b). Only the intraresidual cross-peaks H1'-H6/H8 are marked for clarity. Starting points are marked with blue, end points with red circles. All cross-peaks expected for a regular B-DNA helix are observed for both duplexes.*

4.1.2 Results

4.1.2.1 Chemical shift analysis

The NOESY-spectra were assigned with the sequential approach [Roberts, 1993, Bloomfield et al., 2000]. The sequential assignments of the sugar H1' and base H6/H8 protons for 13merRef and 13mer2AP are shown in Fig. 4.2a and 4.2b, respectively. Cross peaks expected for a regular B-DNA helix are present in the NOESY-spectra of both samples. Based on the assignment of the H1' and H6/H8 protons, the remaining base and sugar protons could be assigned by combining the information from the COSY-, TOCSY-, HMQC-, and NOESY-spectra. Assignment of the exchangeable protons was done in the WATERGATE-NOESY-spectrum. The imino proton of T20 was easily identified, because of the symmetry of the sequence, but the remaining imino, amino, and H2 protons had to be referenced to the already assigned non-exchangeable C H5 protons via the H42/H41-H5 NOE cross-peaks.

^{1}H Chemical Shift Differences (CSDs) between 13merRef and 13mer2AP are negligible (< 0.10 ppm) for all but the H1', imino, and H2 protons of the central three base pairs (see Table 4.1 and Table .13 in the Appendix, section .10). There is no significant trend that either 3'- or 5'-neighbours exhibit larger CSDs. No CSDs for the H6 and H8 protons

Tabelle 4.1: *Selected ^{1}H Chemical shift differences (CSD) (reference HOD at 4.80 ppm, calculated as X(13merRef)-X(13mer2AP)) for 13merRef and 13mer2AP. Only the inner 7 base pairs are shown. In the case of X7 H2 the difference between the chemical shifts of H2 (A) and H6 (2AP) is taken.*

Residue	H1'	H1/H3/H41/H42	H2/H5/H7	H6/H8
G4	-0.01	-0.01	-	-0.03
C5	-0.08	0.03/-0.01	-0.02	-0.04
A6	-0.17	-	-0.16	-0.01
X7	0.29	-	-0.67	0.16
A8	-0.12	-	-0.13	-0.04
C9	0.00	-0.03/0.02	-0.06	-0.04
G10	-0.01	-0.05	-	-0.02
C17	-0.04	-0.01/-0.02	-0.02	-0.02
G18	0.00	0.03	-	-0.03
T19	-0.03	0.21	-0.04	-0.04
T20	0.09	0.53	-0.03	0.01
T21	0.11	0.13	0.00	-0.02
G22	-0.04	-0.05	-	0.00
C23	0.01	-0.02/-0.02	-0.02	0.01

for purine and pyrimidine bases with the exception of residues A7/2AP7 are observed.

In Fig. 4.3 the sum of the absolute values of the CSDs of all protons belonging to one residue is given for 13merRef to 13mer2AP (blue columns) and 13merRef to 13merRefGC (red columns). In 13merRefGC the central base pair of 13merRef is substituted by G:C. With the exception of the modification site, absolute per-residue CSDs from 13merRef to 13mer2AP are smaller than to 13merRefGC. Per-residue CSDs are significant two bases in each direction from the modification site in the 2AP-containing strand. In the unmodified counterstrand only the central three T bases exhibit significant CSDs. The chemical shifts of all assigned protons and carbons for 13merRef and 13mer2AP are given in the Appendix, sections .8 and .9, respectively.

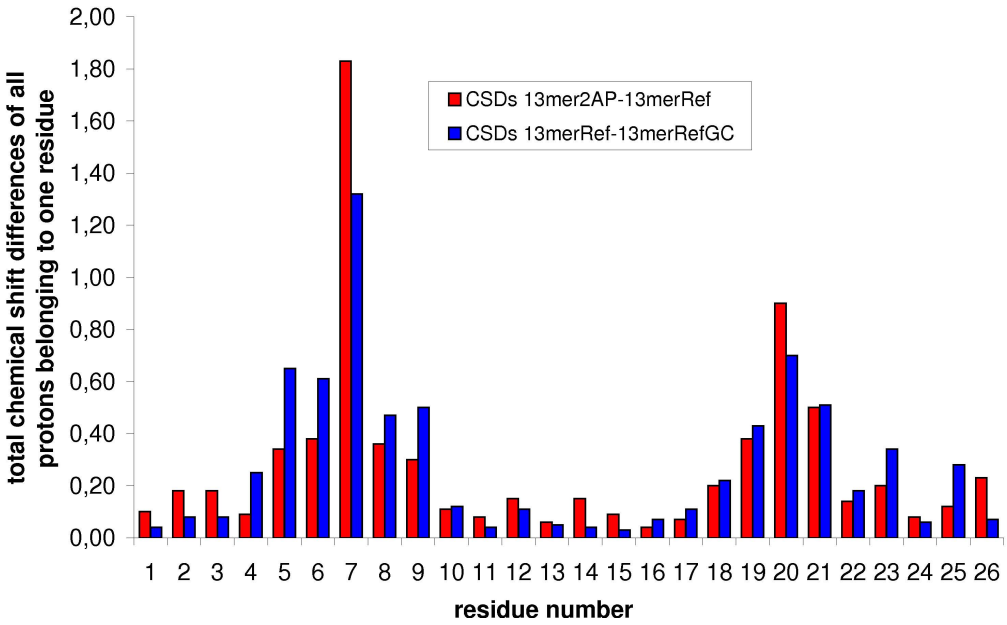


Fig. 4.3: *The sum of the absolute values of the CSDs of all protons belonging to one residue is given for: 13mer2AP to 13merRef (blue columns) and 13merRef to 13merRefGC (red columns) for comparison.*

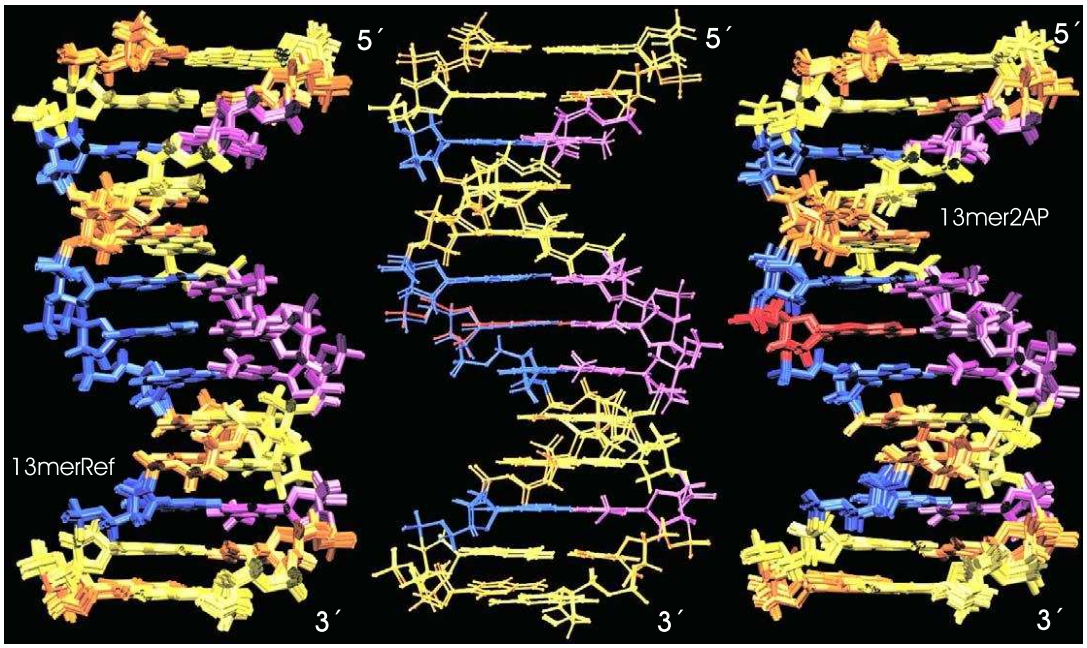
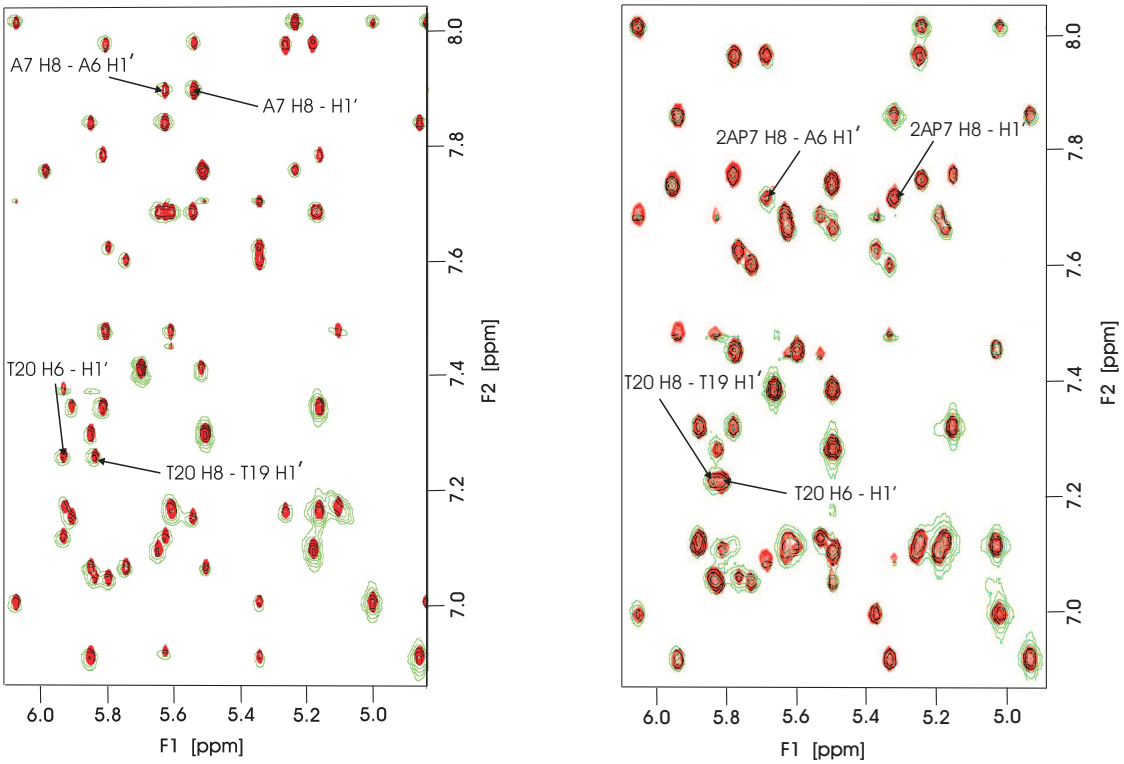


Fig. 4.4: *Overlay of the 10 best-energy, minimum-violation structures for 13merRef and 13mer2AP. The RMSD among each set of structures is 0.30 Å and 0.33 Å, respectively. The average structures for the two duplexes are compared in the middle; their RMSD is 0.46 Å.*

4.1.2.2 Structural comparison

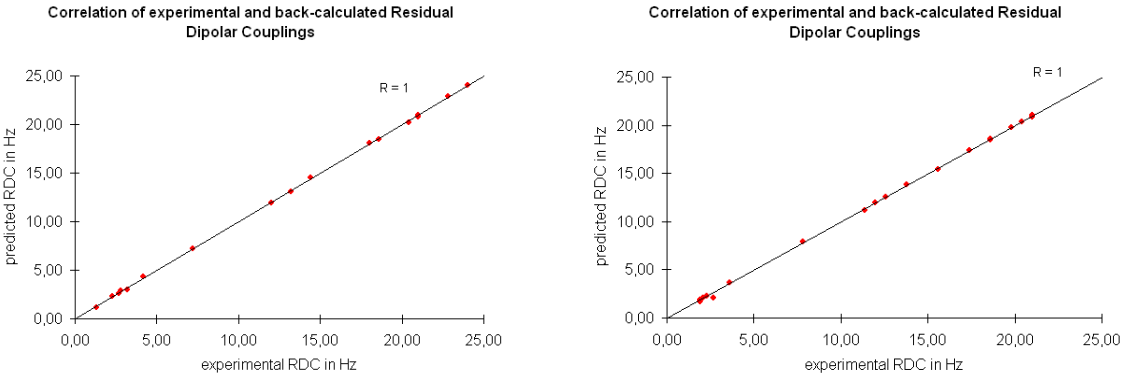
From a family of 100 calculated structures the 10 minimum-energy, violation-free structures are shown in Fig. 4.4 for 13merRef (left) and 13mer2AP (right). The latter were used to calculate an average structure each. The accuracy of the calculations was checked by back-calculating the NOESY-spectrum from the average structure, followed by comparison with the experimental spectrum (Fig. 4.5a and 4.5b). Additionally, the RDCs were back-calculated from the average structure with the program PALES [Zweckstetter, 2008]. The correlation plots of experimentally determined vs predicted RDCs are shown in Fig. 4.6a and 4.6b and yielded correlation factors (R) of 1.000 and q-factors of 0.002 and 0.003 for *13merRef* and *13mer2AP*, respectively. The precision of the calculations can be assessed by the RMSD of the 10 best structures from their average, 0.30 Å for 13merRef and 0.33 Å for 13mer2AP. An overlay between the averaged structures is shown in Fig. 4.4 (middle part); their RMSD is 0.46 Å.

Helical parameters were calculated for the 10 minimum-energy, violation-free structu-



(a) *13merRef* **(b)** *13mer2AP*

Fig. 4.5: *Overlay of the experimental NOESY-spectrum at 150 ms mixing time (green) and the NOESY-spectrum back-calculated from the average structure (red). Arrows point to NOE cross-peaks involving the modification site.*



(a) *13merRef* **(b)** *13mer2AP*

Fig. 4.6: *Plot of the experimental vs predicted RDCs for 13merRef and 13mer2AP.*

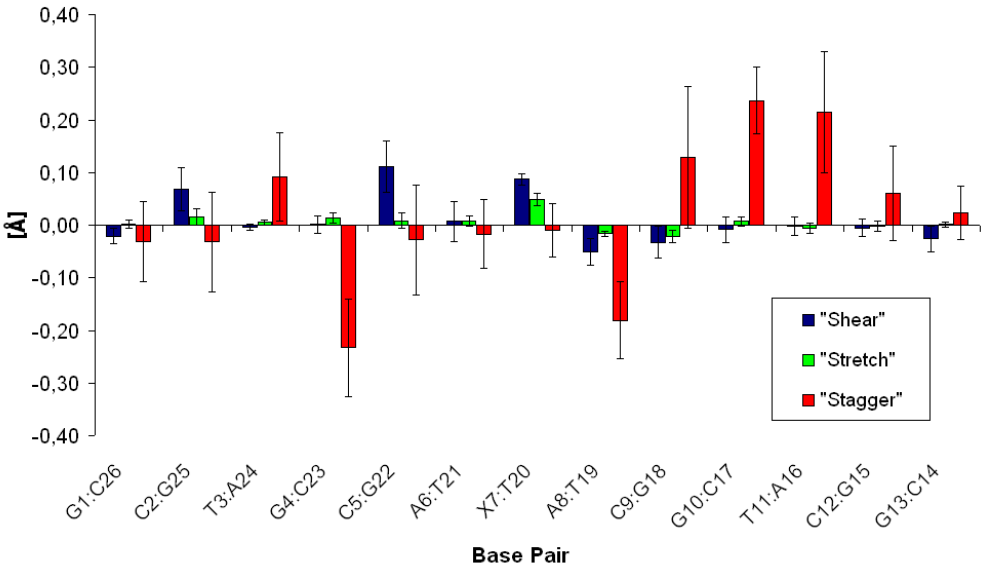


Fig. 4.7: *13merRef and 13mer2AP: Differences (X(13merRef)-X(13mer2AP)) in translational helical parameters between base pair partners [Å]. Blue columns represent "Shear"-values, green ones "Stretch" and red ones "Stagger". Error bars indicate the estimated uncertainty.*

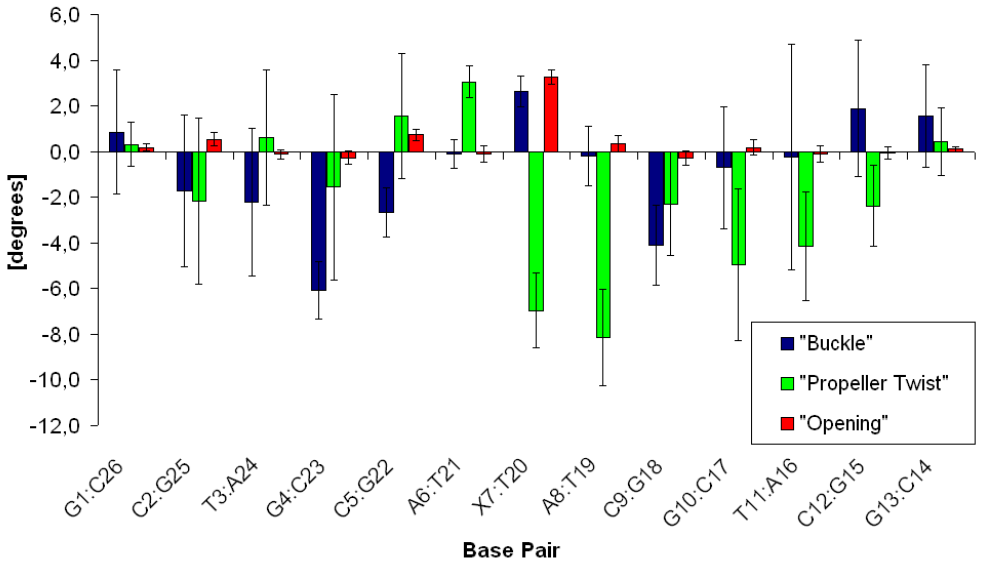


Fig. 4.8: *13merRef and 13mer2AP: Differences (X(13merRef)-X(13mer2AP)) in rotational helical parameter between base pair partners [°]. Blue columns represent "Buckle"-values, green ones "Propeller Twist" and red ones "Opening". Error bars indicate the estimated uncertainty.*

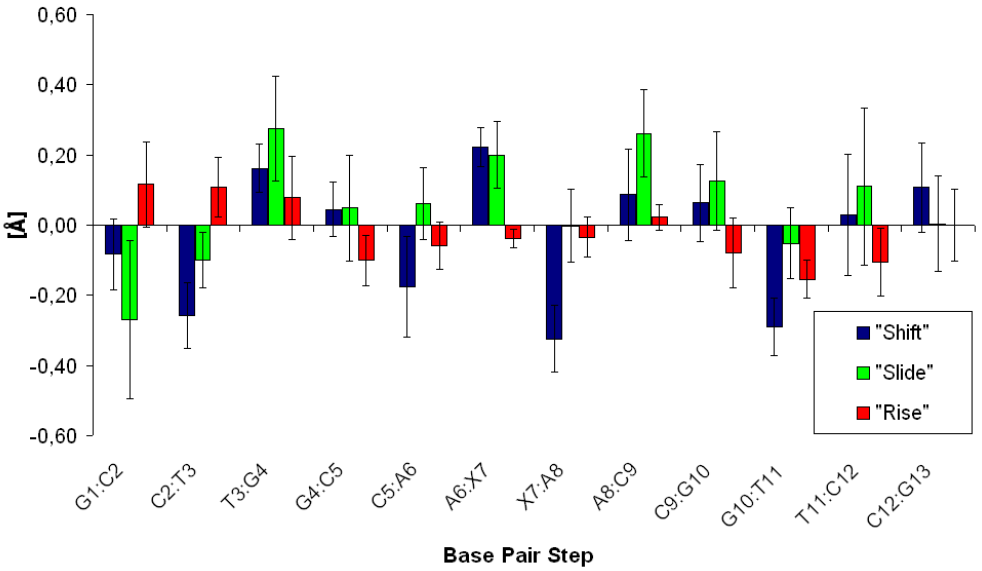


Fig. 4.9: *13merRef and 13mer2AP: Differences (X(13merRef)-X(13mer2AP)) in translational helical parameter between base pairs [Å]. Blue columns represent "Shift"-values, green ones "Slide" and red ones "Rise". Error bars indicate the estimated uncertainty.*

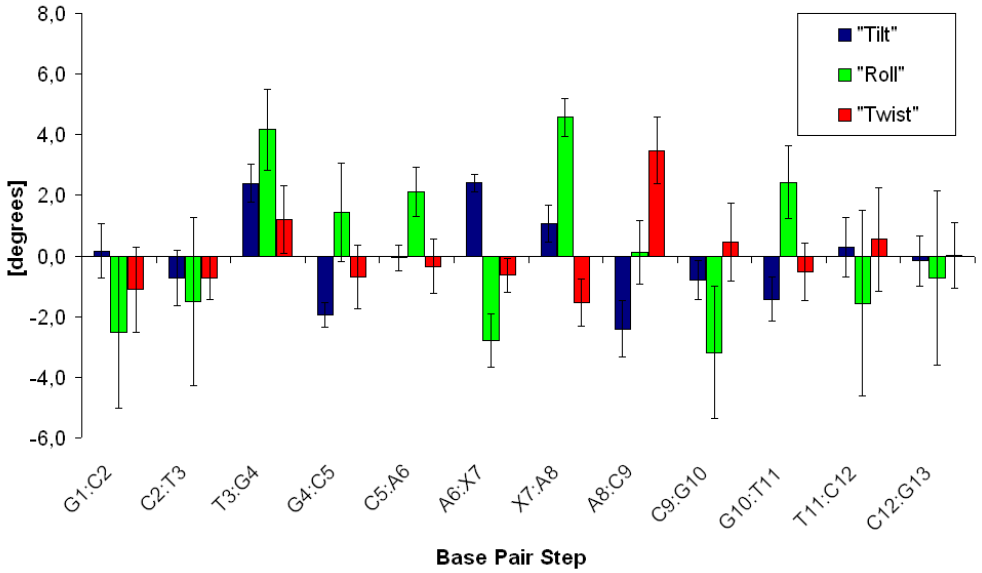


Fig. 4.10: *13merRef and 13mer2AP: Differences in rotational helical parameter between base pairs [°]. Blue columns represent "Tilt"-values, green ones "Roll" and red ones "Twist". Error bars indicate the estimated uncertainty.*

res of 13merRef and 13mer2AP with the program 3DNA [Lu and Olson, 2003]. Fig. 4.7 and 4.8 depict differences between 13merRef and 13mer2AP in translational and rotational helical parameters between base pair partners, while Fig. 4.9 and 4.10 display differences in translational and rotational helical parameters between base pairs. The value (X) represented by a column in Fig. 4.7- 4.10 is calculated as:

$$X = \frac{1}{10}\left(\sum_{n=1}^{10} X_n(13merRef) - \sum_{n=1}^{10} X_n(13mer2AP)\right) \tag{4.1}$$

where $X_n(13merRef)$ and $X_n(13mer2AP)$ represent the helical parameter values calculated for the 10 minimum-energy, violation-free structures of 13merRef and 13mer2AP, respectively. The RMSD of these values to their average is shown as an error bar. Thus a measure for the precision of a given helical parameter is obtained. In general, for translational parameters only values which deviate by at least 0.1 Å (with the corresponding uncertainties taken into account) and for rotational parameters $> 5\,^\circ$ are interpreted. Deviations below these thresholds are too weak to be reliably described by the force field (deviations of 0.1 Å and $5\,^\circ$ from the equilibrium value are allowed for bond lengths and angles respectively).

In the following, the observed perturbations in helical parameters are presented. For definition of the helical parameters please see section 2.1, Fig. 2.5.

- The "Stagger"-values (red columns in Fig. 4.7) differ significantly for base pairs G4:C23 (-0.23 ± 0.09 Å), A8:T19 (-0.18 ± 0.07 Å) and G10:C17 (0.24 ± 0.06 Å), while values for "Shear" and "Stretch" (blue and green columns in Fig. 4.7) do not show significant deviations.

- Of the rotational helical parameters within base pairs only the "Propeller Twist"-values (green columns in Fig. 4.8) of base pairs X7:T20 (-7.0 ± 1.6 °) and A8:T19 (-8.1 ± 2.1 °) exhibit significant deviations, while "Buckle"- and "Opening"-values are equal within the estimated error (blue and red columns in Fig. 4.8 respectively).

- The rotational helical parameters between base pairs "Tilt", "Roll" and "Twist" (blue, green and red columns in Fig. 4.10 respectively) do not show significant variations within

the estimated error.

- The "Shift"-values (blue columns in Fig. 4.9) differ significantly for base pair steps C2:T3 (-0.26 ± 0.10 Å), A6:X7 (0.20 ± 0.03 Å) and A8:C9 (0.26 ± 0.03 Å). The "Slide"-values (green columns in Fig. 4.9) differ significantly for base pair steps T3:G4 (0.28 ± 0.15 Å), A6:X7 (0.22 ± 0.11 Å) and A8:C9 (-0.26 ± 0.12 Å). The "Rise"-values (red columns in Fig. 4.9) do not differ within the estimated confidence interval.

In summary, significant deviation in helical parameters are observed throughout the duplex, with the central three base pairs exhibiting the largest deviations. The average helical parameters calculated from the values for the 10 minimum-energy, violation-free structures of 13merRef and 13mer2AP are listed in the Appendix in sections .11 and .12, respectively.

4.1.2.3 Comparison of base pair dynamics

The T20 imino proton resonance behaves substantially different depending on the counter base 2AP or A. In 13mer2AP this resonance can be observed in the 1D-spectrum, though it is broadened substantially (cf. Fig. 4.12). The corresponding diagonal signal in the NOESY-spectrum however vanishes with increasing mixing time beyond 100 ms. Fig. 4.11 illustrates this unusual decay behaviour by comparing signal intensities for the diagonal and representative cross-peak signals of T20 (•) and T3 (▲) in 13mer2AP. Diagonal signals T20 H3 and T3 H3 (solid lines) are normalized at 50 ms to show their different decay behaviour. Without this normalization, the diagonal peak of T20 H3 reaches only 20 % of the T3 H3 signal intensity at 50 ms. Cross-peaks T20H3–2APH6 and T3H3–A24H2 (dashed lines) are given relative to the corresponding diagonal signal at 50 ms. The diagonal T20 H3 peak almost vanishes when going from 50 to 100 ms mixing time. The intensity of the NOE cross-peak to 2AP H6 decays slowly, in contrast to the T3H3–A24H2 cross-peak where the intensity increases for the first 200 ms.

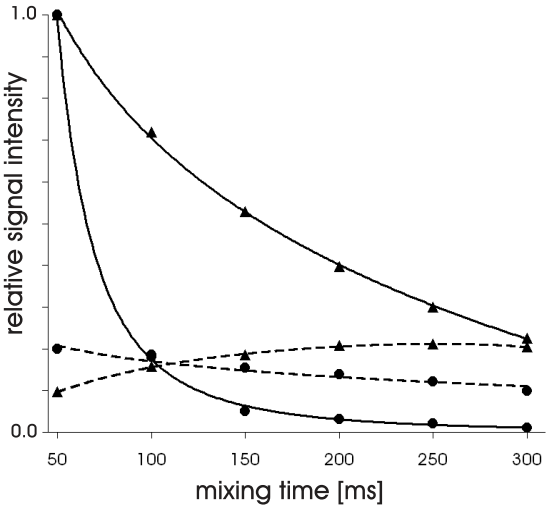


Fig. 4.11: *T20 (•) and T3 (▲) imino proton intensities for 13mer2AP at 298 K. Solid lines depict diagonal signals, while dashed lines represent cross-peak intensities of T20H3–2APH6 and T3H3–A24H2.*

To test whether H_2O exchange is responsible for the unusual decay behaviour of T20 H3 we performed water saturation transfer experiments. In the latter experiments 1D-spectra in H_2O are aquired with two different methods of attenuating the water signal. With the presaturation method the water signal is saturated before the actual pulse sequence (Fig. 4.12 (B,D): irradiation time 3 s, strength 55 dB). Consequently, exchange of now saturated water protons with unsaturated imino protons leads to attenuation of the latter signal. This cosaturation can be circumvented by using the WATERGATE

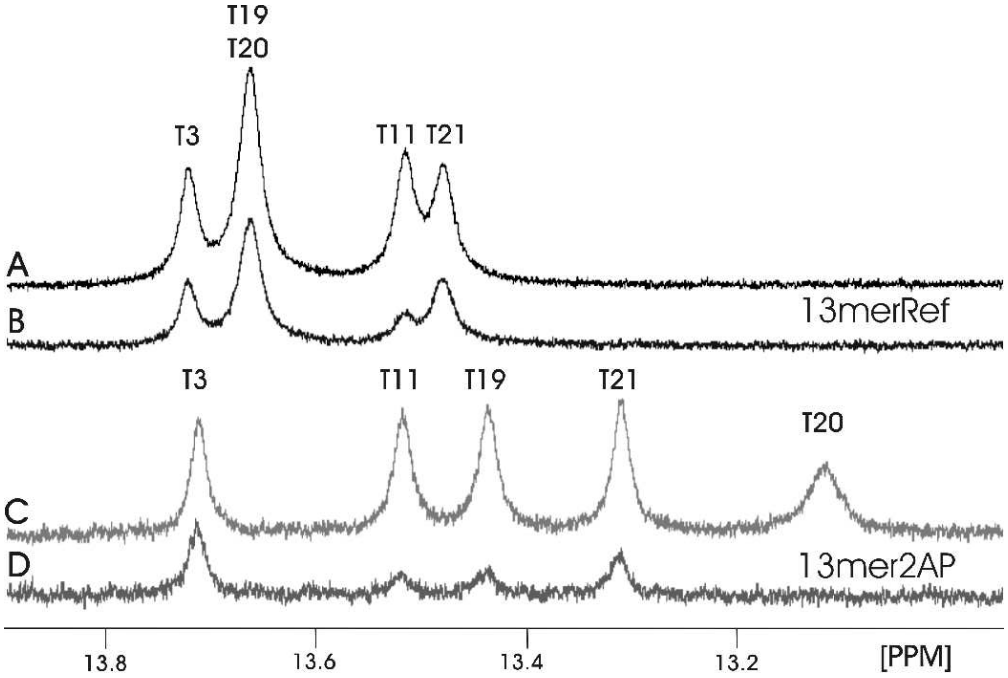


Fig. 4.12: *Saturation transfer experiments in H_2O. Resonances from exchanging protons are reduced in the 1D presaturation spectra (lower black lines, B,D), reflecting the relative rates by which imino protons exchange with the solvent. Water saturation is avoided in 1D WATERGATE spectra which are shown for comparison (upper gray lines, A,C).*

pulse sequence (Fig. 4.12 (A,C)), which does not excite the water protons and thus prevents their detection. Here water exchange with imino protons leaves their signal unperturbed. Fig. 4.12 depicts the imino proton region with both attenuation techniques, presaturation (B,D) and WATERGATE (A,C) for 13merRef, 13mer2AP respectively. In 13mer2AP the cosaturation of the T20 imino proton is so strong that the signal is not observable in presaturation experiments. The signals of the directly adjacent bases T19 and T21 are reduced in intensity when compared to the corresponding signals in 13merRef.

Base pair dynamics can be studied by measuring the base pair lifetime τ_{op}. The latter is obtained by extrapolating a plot of imino proton exchange τ_{ex} vs inverse base concentration 1/[B] to infinite catalyst concentration (for explanation see section 2.2.1). Fig. 4.13 shows the corresponding data and linear fits for the central 7 base pairs and the extrapolated lifetimes for 13merRef and 13mer2AP. Lifetimes in green refer to overlap-

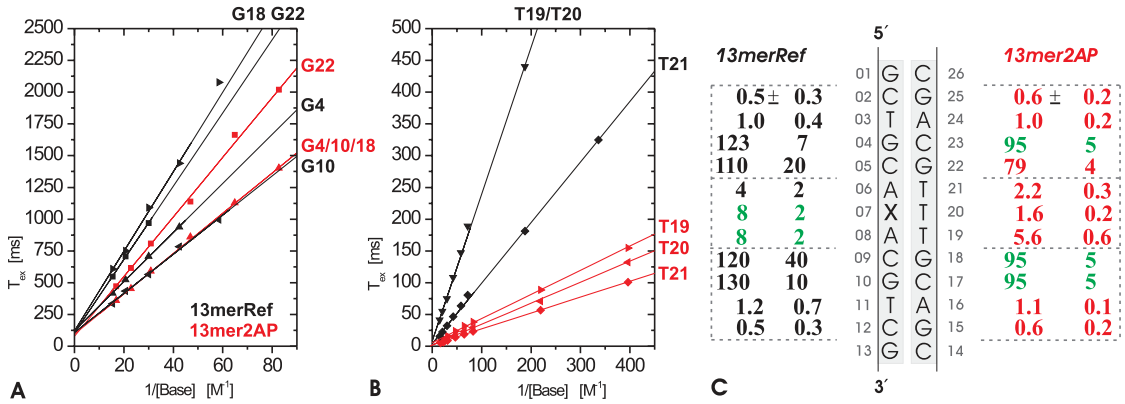


Fig. 4.13: *Basepair lifetime determination. The inversion recovery of imino proton signals is affected by TRIS base which catalyzes exchange with water. The exchange time τ_{ex} depends on the inverse base concentration 1/[B], with different ranges for G:C and A:T pairs. Extrapolation to infinite concentration gives the lifetimes which are collected in the right panel. Lifetimes in green could not be determined separately due to spectral overlap.*

ped resonances in the imino proton region. Their overlap is complete and signal recovery identical; therefore only averaged lifetimes can be given. The R^2-values for all linear fits are above 0.996. Confidence intervals for each lifetime are also determined from the fits. The lifetimes of the terminal base pairs could not be measured due to "base pair fraying" which is commonly observed at the helical termini. Here base pair lifetimes are considerably shortened, broadening the signal from terminal imino protons to the point of vanishing and weakening the signal from neighbouring ones [Leijon and Graslund, 1992, Nonin et al., 1995]. Lifetimes for the semiterminal G:C (0.5 ms) and A:T base pairs (1.1 ms) are found to be identical for 13merRef and 13mer2AP within the estimated error (the corresponding linear plots are shown separately in Fig. 4.15 for reasons of clarity). Contrary to the outer base pairs, lifetimes for the inner seven pairs differ between 13merRef and 13mer2AP. The substitution A→2AP reduces τ_{op} severely for the central A:T pairs (by factors 0.55, 0.20 and 0.70 for T21, T20, T19) and less so for the more remote G:C pairs (0.8-0.7).

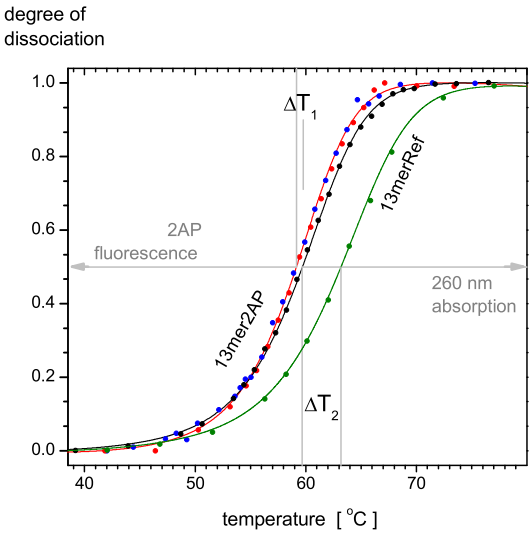


Fig. 4.14: *Melting curves via 13mer2AP absorbance (black) and 2AP fluorescence yield (blue points - after heating, red points - after cooling).*

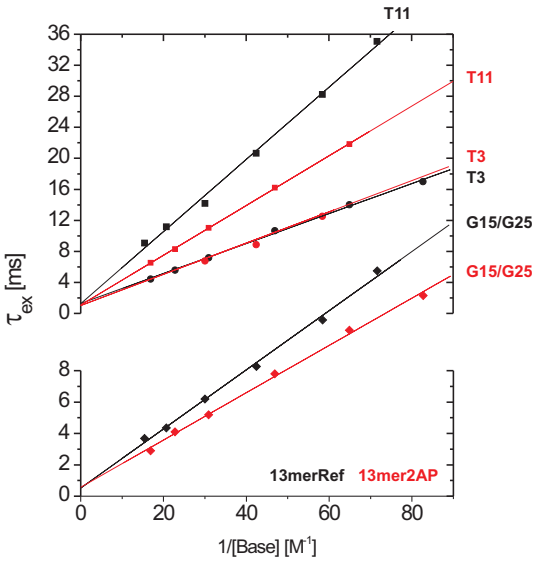


Fig. 4.15: *Linear fits for the imino proton exchange times of T3,T11,G15 and G25 vs the inverse base catalyst concentration.*

4.1.2.4 Comparison of duplex melting

Melting curves of 13mer2AP are shown in Fig. 4.14. Black points depict the melting point as determined by following the temperature-dependent absorption at 260 nm. Blue and red points represent duplex melting as determined by monitoring temperature-dependent 2AP fluorescence yield for heating and cooling, respectively. With UV absorption the melting behavior of the entire duplex is monitored whereas the 2AP fluorescence yield is sensitive to the local environment only. Melting points of 59.7 and 59.2 °C for the duplex as a whole and the centre are measured respectively. Thus $\Delta T_1 = 0.5\,\mathrm{K}$ can be determined for duplex vs centre melting. The melting point of 13merRef was measured as 63.2 °C, with $\Delta T_2 = 3.5\,\mathrm{K}$ for 13merRef vs 13mer2AP melting.

4.1.3 Discussion

Structure perturbation

Nordlund et al. [1989] studied 2AP in a palindromical decamer sequence. They reported that NOE cross-peaks expected for a regular B-DNA helix, were missing to the 5'-side of the 2AP incorporation site. From that they conclude that the structure is perturbed locally with the perturbation extending only to the adjacent base pairs [Nordlund et al., 1989]. In contrast, all NOE cross-peaks expected for a regular B-DNA helix have been identified for 13mer2AP. This indicates that 2AP incorporation induces no pronounced perturbation in 13mer2AP. A possible explanation might be the higher detection limit of the 600 MHz NMR spectrometer (instead of 300 MHz) and the 5-fold higher duplex concentration employed in this work. Another reason might be the low stability of the decamer duplex, whose melting point decreases by 8.6 K (to 32.8 °C) upon introduction of the two 2AP residues [Nordlund et al., 1989].

The per-atom CSD data analysis for 13merRef and 13mer2AP (cf. section 4.1.2.1) supports the above conclusion that no pronunced perturbation is induced upon 2AP incorporation. Significant per-atom CSDs are observed exclusively between protons spatially adjacent to 2AP in 13mer2AP or A in 13merRef. This suggests that the observed CSDs are due to the different ring current effects induced by 2AP and A [Wijmenga et al., 1997]. Thus per-atom CSD data does not hint at structural differences between the two samples, but instead points to electronic differences between 2AP and A, which are evidenced by quantum chemical calculations [Broo, 1998, Mishra et al., 2000, Jean and Krueger, 2006].

On the contrary, absolute per-residue CSDs indicates that also structural differences exist between 13merRef and 13mer2AP which propagate at least two base pairs in each direction from the modification site. Comparison with the total CSDs between 13merRef and 13merRefGC suggests that the substitution of an A:T by a G:C base pair has a stronger impact on the helical structure than substitution of A by 2AP since total CSDs for the latter are equal or less for the base pairs adjacent to the modification site (cf. Fig. 4.3). This indicates that the helical structures of 13merRef and 13mer2AP both

adopt a regular B-DNA conformation. Yet very subtle structural changes between these two structures exist, which effect the significant CSDs observed for the bases C5 and C9.

A comparison to CSD data on other single mismatch sites is instructive (Note that ^{1}H-CSD data for A→2AP substitution, other than reported here, is not available.). Bhattacharya et al. [2002] examined the effect of single mismatches A:A, G:G, C:C on the imino proton chemical shift. CSDs for the adjacent base pairs were significantly more perturbed, than in our case, reflecting the disturbed helical structure resulting from the non-WC-geometries of the mismatch sites. Klewer et al. [2000] studied incorporation of 3-nitropyrrole into DNA. They report CSDs to the unperturbed structure that are comparable to these of 13merRef/13mer2AP. This is surprising given the fact that 3-nitropyrrole is in a syn conformation. Despite this perturbation, the overall helical arrangement is found to be the B-form. This is in line with the results for 13mer2AP, where a B-type helix with small but significant deviations to the structure of 13merRef is suggested by the CSD data.

The overall B-DNA helical structure of 13merRef and 13mer2AP is supported by the calculation results. Slightly smaller RMSDs among the 10 best-energy structures (0.30 and 0.33 Å for 13merRef and 13mer2AP) as compared to the RMSD between the corresponding average structures (0.46 Å) are observed. This indicates that while the overall conformation is identical, minor but significant differences exist. These differences can be visualized by analysing and comparing the helical parameters for 13merRef and 13mer2AP. All of the helical parameters are within the range typically observed for B-DNA (cf. section 2.1). However, significant deviations between 13merRef and 13mer2AP structures exist throughout the duplex (see section 4.1.2.2) for the parameters “Stagger” and “Propeller Twist” between base pair partners, and “Slide” and “Shift” between two base pairs. The largest deviations are observed for the central three base pairs. This is shown in Fig. 4.16 which displays the latter pairs enlarged and from a tilted angle in order to improve visualization of the helical parameters of interest. In conclusion, the structural differences between 13merRef and 13mer2AP are small but detectable.

The structural perturbation induced upon 2AP incorporation is very weak compared

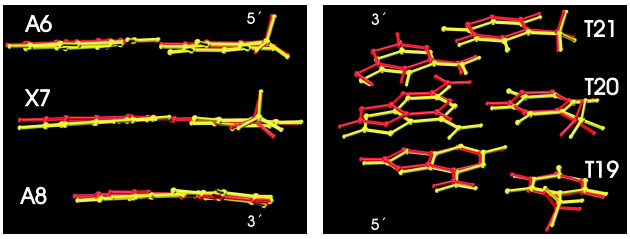


Fig. 4.16: *Comparison of the three central base pairs (average structures, center of Fig. 4.4 enlarged) for 13mer2AP (yellow) and 13merRef (red). The backbone was omitted for clarity. In the right panel the view was tilted to better visualize the differences in "Shift"- and "Slide"-values.*

to other base analogues. Engman et al. investigated the structure of tC containing DNA. They find that the overall B-DNA conformation is preserved. However, the DNA is slightly bent and several NOE cross-peaks involving tC are not consistent with a B-DNA conformation. Furthermore, the second ring of tC, which is not involved in base pairing, extends into the major groove, thus preventing protein docking [Engman et al., 2004]. An even larger fluorophore, pyrene, was used by Smirnov et al. [2002] as a base pair analogue opposite to an abasic site. The overall B-DNA conformation is again preserved, but local mobility or even an alternative orientation of the 5'-adjacent base pair is introduced. This is indicated by the presence of two imino proton signals and very weak or lacking NOE cross-peaks for this base pair [Smirnov et al., 2002]. 2,4-Difluorotoluene - a steric mimic of T devoid of hydrogen bonding sites - was introduced by Guckian et al. [1998]. Although a strong thermodynamic destabilization is found (ΔT=11 K), no significant deviation of B-DNA conformation is observed as indicated by uninterrupted sequential connectivities. However, a detailed analysis of helical parameters and base pair dynamics is lacking. A whole base pair devoid of hydrogen bonding was used in a related study [Guckian et al., 2000]. It was demonstrated that overall B-DNA conformation is retained. Local mobility however is increased as sensed by broadening of the imino proton resonance at position 5. None of these works use a joint (structure, thermodynamics and base pair kinetics) approach to study perturbation upon incorporation of fluorophores. Moreover, a detailed analysis of helical parameters and a comparison with a control with WC base pairs only is lacking. Thus the present work constitutes a novel approach to the topic of DNA perturbation studies.

Basepair lifetimes

Although the surrounding structure in 13merRef and 13mer2AP is almost identical, large dynamic differences are indicated by the unusually fast decay of the T20 imino proton diagonal signal in the NOESY-spectrum of 13mer2AP (cf. Fig. 4.11). Inspection of the diagonal signal in NOESY spectra was used by Engman et al. [2004] to qualitatively check for increased water exchange in a modified DNA duplex. The lack of any uncommon phenomena led to the conclusion that base pair dynamics are not perturbed by

incorporation of the fluorophore tC. Following that reasoning, the fast decay observed with the T20 imino proton may indicate increased chemical exchange with water. This interpretation is corroborated by the results of the water saturation transfer experiments. Here the signal of the T20 imino proton is completely cosaturated in 13mer2AP, while the signals from T3 in the same duplex and T20 in the reference duplex are only slightly reduced (Fig. 4.12). Interestingly though, intensities of the adjacent imino protons (T19,T21) are more reduced compared to 13merRef, which indicates faster water exchange for these protons, too. This in turn suggests that 2AP incorporation also effects the dynamics of the adjacent base pairs.

The latter hypothesis has been validated by the results from base pair lifetime measurements. Upon 2AP incorporation the lifetimes of the central seven base pairs seem to be reduced. But the lifetimes of the G4, G10 and G18 imino protons for 13mer2AP and T19 and T20 for 13merRef (shown in green in Fig. 4.13) could not be determined separately due to complete spectral overlap. However their lifetimes should be similar since their recovery behaviour is identical (as assessed by inspection of the 21 1D-spectra resulting from the 21 delay settings after the inversion pulse). Thus the averaged lifetimes can be interpreted for the overlapped protons. Interpretation of differences in base pair lifetimes for the G18, T19 and T21 imino protons of 13merRef and 13mer2AP is complicated as the estimated confidence intervals overlap slightly. However, differences between these lifetimes can be assumed due to the observed reduction of imino proton signal intensity in the water saturation transfer experiment. Additionally, significantly reduced lifetimes for the G4 and G10 imino protons indicate an extended effect of 2AP incorporation on the base pair dynamics of the three adjacent base pairs in each direction. An alternative explanation for the reduced base pair lifetimes of G4 and G10 might be different sample conditions for 13merRef and 13mer2AP. This can be a significant source of errors, when comparing two different samples. However, lifetimes of the base pairs C2:G25, T3:A24,T11:A16, and C12:G15 are equal within the estimated error although these base pairs are influenced by base pair fraying effects. Since the latter effect is sensitive to solution properties (pH, buffer, temperature), different sample conditions

can be ruled out as an explanation. Additionally, the excellent agreement between these lifetimes shows that the lifetime measurements are reliable even below 1 ms. In summary, one can conclude that 2AP incorporation has an extensive, distributed effect on the base pair dynamics of the three adjacent pairs in each direction.

Base pair lifetimes in 2AP-containing DNA have been examined by Lycksell et al. [1987]. Their result for the modification site, of 1 ± 2 ms, agrees qualitatively with the one reported here but is less precise. In contrast, these authors do not observe an extensive, distributed effect on base pair dynamics due to the incorporation of 2AP into the DNA helix. In their palindromic decamer sequence, the melting point is lowered upon incorporation of 2AP from 41.4 °C to 32.8 °C [McLaughlin et al., 1988]. This suggests that at 25 °C, the temperature at which base pair lifetimes were measured, the latter are already affected by duplex melting. This is corroborated by the fact that the opening motion of some inner base pairs is too rapid to be observed. Further support stems from the fact that the central two A:T pairs in their reference sequence constitute an A-tract motif, which would suggest a base pair lifetime for the core A:T pair of over 10 ms [Leroy et al., 1988, Moe and Russu, 1990] rather than 6 ms as observed by Lycksell et al. [1987]. Its length of 13 base pairs makes the non-palindromic duplex studied in the present work thermodynamically more stable. Thus it is less susceptible to destabilization due to base pair fraying. Influence of non-native modifications on the base pair dynamics of adjacent base pairs has been reported in the literature. Moe and Russu [1992] investigated a palindromic dodecamer sequence, with a G:T mismatch introduced at position 4. An increased base pair opening rate is demonstrated for position 5. A similar effect at position 3 could not be resolved due to the high error for the obtained base pair lifetime (60 %) in the reference and signal overlap in the mismatch sequence [Moe and Russu, 1992]. These observations support the finding that 2AP incorporation effects the base pair lifetimes of the three adjacent pairs in each direction.

The extensive, distributed effect of 2AP incorporation on base pair opening dynamics can be explained with transition state theory. For base pair opening to occur, the stabilizing enthalpy contribution from the hydrogen bonds between the partners and the

stacking interaction with the adjacent base pairs have to be overcome when the transition state is formed. Thus a reduction in strength of either interaction would favor base pair opening and thereby lead to a reduced base pair lifetime. Similar reasoning is used to explain the severely reduced base pair lifetimes of the helical termini [Nonin et al., 1995].

Reduced stacking interactions and hydrogen bonding energies throughout the duplex are indicated by the 3.5 K reduction in overall duplex melting temperature upon 2AP incorporation. This is supported by the results of the structural analysis. The higher melting point suggests that stacking interactions and hydrogen bonding in 13merRef are stronger than for 13mer2AP. Thus any change of the position of base pairs (stacking) or base pairing partners (hydrogen bonding) relative to each other must lead to the reduction of either stabilizing energy term. Consequently, the observed deviations in "Stagger" and "Propeller Twist" (defined between base pair partners) on the one hand and "Shift" and "Slide" (defined between base pairs) on the other hand indicate reduced hydrogen bonding and stacking interactions, respectively. Locally, the strong impact of 2AP incorporation on the central three base pairs is indicated by the 0.5 K reduction in duplex melting temperature when monitored via 2AP fluorescence. Since the latter is sensitive only to the directly adjacent base pairs, the observed reduction implies considerable premelting and thus destabilization of the duplex structure around the 2AP modification site as a whole. Premelting around the incorporation site has also been observed by Law et al. [1996] in a 2AP modified undecamer, where the destabilization was found to be even stronger (1.6 K). As pointed out before, deviations in helical parameters are strongest for the central three base pairs, which corroborates the results of the melting study. The latter results are further supported by the fact, that base pair lifetimes are considerably shortened and water saturation transfer is increased for the central three pairs. Thus one can conclude that 2AP incorporation destabilizes the duplex structure, by reducing the stacking and hydrogen bond interactions. This in turn leads to a lower activation enthalpy for the transition state of the base pair opening reaction. Thereby the extensive distributed effect of 2AP incorporation on the base pair lifetimes can be

explained.

The effect of 2AP incorporation on the base pair dynamics of remote pairs can be used to explain results from DNA-protein interaction studies. The latter indicate that although protein activity is reduced, binding affinities are enhanced upon incorporation of 2AP into DNA [Brennan et al., 1986, Petrauskene et al., 1995, Malygin et al., 1999, Reddy and Rao, 2000]. Methyltransferases bind to DNA by flipping the target base out of the helix [Allan and Reich, 1996, Allan et al., 1998, Malygin et al., 1999, Reddy and Rao, 2000]. Thus higher base pair opening rates would enhance this binding process. The latter assumption is supported by the observation of binding enhancement upon introduction of mismatches [Moe and Russu, 1992]. Reddy and Rao [2000] investigated EcoP15I DNA Methyltransferase binding to DNA. They found that binding was enhanced for 2AP-containing DNA as compared to native DNA, regardless, whether 2AP replaces A in the recognition sequence or outside. Similar results were reported by Malygin et al. [1999] for the T4 DAM Methyltransferase. Enhancement of the binding process when 2AP is substituted for an A just outside the recognition center can only be explained by the influence of 2AP substitution on the base pair opening dynamics of several pairs in each direction as was observed for 13mer2AP. Similar observations were made with 2AP-containing DNA binding to restriction endonucleases [Petrauskene et al., 1995, Brennan et al., 1986], which can be explained analogously.

However, the reduced base pair lifetime of 2AP:T cannot account for the increased water exchange in the absence of added catalyst. Despite the fact that in 13mer2AP the base pair lifetimes of A16:T11 (1.1 ms) and A24:T3 (1.0 ms) are significantly smaller than for 2AP:T (1.6 ms), the corresponding imino resonances are still detectable in the presaturation experiment, while that of 2AP:T is missing (see Fig. 4.12). This observation suggests that the short base pair lifetime of 2AP:T accounts only in part for the increased water exchange. Since τ_{op} is measured in the limit of high catalyst concentration, but the presaturation experiments were carried out in the absence of catalyst, intrinsic catalysis seems to be involved. One possible catalytic site could be the N1 atom in 2AP, which may have higher basicity compared to A. Electronic differences are indeed

indicated by the CSD data, but it is unlikely that the pK is raised sufficiently high to account for the results. An alternative mechanism involves the T20 O4 atom, which lacks a hydrogen bond to its partner base and can be easily accessed by solvent or base catalyst via the major groove. Thus, by forming a strong hydrogen bond with a potential, effective catalyst, it brings the latter within reach of the imino proton. In this way the catalytic ensemble is preorganised, increasing the probability of the catalytic exchange. Additionally, lack of the sterically demanding amino group in the major groove suggests that for the imino proton to be accessed by the catalyst, full opening of the 2AP:T base pair is not required. Thus exchange catalysis is more effective for 2AP:T as compared to A:T base pairs. Support for this explanation comes from a comparison of T_1-values in the absence of added catalyst. The latter is shorter by roughly a factor three or more for T20 in 13mer2AP (29.5 ms) when compared to the other T imino protons in the duplex (T3: 199.7 ms, T11: 83.6 ms,T19: 98.8 ms, T21: 141.0 ms).

Another possible explanation for our observations would be exchange from the closed state. Arguments against this possibility are given by Nonin et al. [1995] who find that exchange from the closed state is negligible even for terminal base pairs, since the imino protons are not accessible sideways. The increased exchange rates of terminal imino protons are explained by reduced stacking interactions [Nonin et al., 1995]. Leroy et al. [1993] find that in C:C^+ mispairs the amino protons of C have different exchange times, depending on whether or not they are hydrogen bonded. This supports the conclusion that exchange from the closed state is strongly inhibited when the imino protons are not accessible sideways. The latter is true however for G:T mismatches, where possible exchange from the closed state was discussed by Moe and Russu [1992]. Since sideway accessibility is not the case for 2AP:T base pairs, exchange from the closed state can be ruled out.

The conclusion that the hydrogen bond of the T O4 atom with bulk water or base catalyst increases the efficiency of imino proton exchange is supported by results of Strekowski et al. [1987]. They studied the interaction of DNA with different intercalators and minor groove binders. Regardless of G:C or A:T binding specificity, all molecules contai-

ning an amino side chain substituent selectively catalyzed A:T imino proton exchange with bulk water. Analogously to the 2AP:T case, this A:T specificity of can be explained by the formation of a hydrogen bond between the amino side chain of the drug and the T non-hydrogen-bonded O2 atom located in the minor groove. The possibility for this interaction is lacking in G:C base pairs since the formation of three hydrogen bonds leaves no hydrogen-bonding partner available. Thus the formation of this hydrogen bond preorganizes the catalytic ensemble, analogously to what was described for 2AP:T.

4.2 2-Hydroxy-7nitrofluorene

4.2.1 Introduction

HNF is a fluorene derivative which has been synthesized and incorporated into a DNA double strand only recently [Dallmann et al., 2009]. Consequently, no reports on HNF or HNF-DNA constructs can be found in the literature. For 2AP subtle changes in structure and dynamics had to be analyzed in order to answer the question of applicability to biological systems. In contrast, HNF has been designed for the proof-of-principle that macromolecular vibrational modes can be measured via transient absorption spectroscopy with polarity probes [Dallmann et al., 2009]. Thus the focus of this part of the work is on the position of the fluorophore inside the DNA helix. A comparison of helical parameters with a native structure (as in case of 2AP) is not instructive since HNF is not as close to a natural base pair as 2AP:T.

Compared to 2AP, HNF is much larger in size and thus functions as a base pair surrogate. Due to synthetic reasons the HNF could only be obtained in sufficient purity in the α-glycosidic form. Preliminary molecular modelling of the HNF-containing duplex with the program HyperChem v7.5 indicated that the hydroxyl linkage introduces orientational flexibility of the HNF moiety. Thus with both, α- and β-glycosidic form of the HNF nucleotide, stacking of the HNF with its adjacent base pairs can be realized. The same test calculations suggested that the introduction of an abasic site imposes less steric strain on HNF and prevents the latter (or its partner base) from being flipped out of the helical stack. Thus the same sequence as for 13mer2AP was studied, with the central base pair substituted by **X**=HNF in the left-

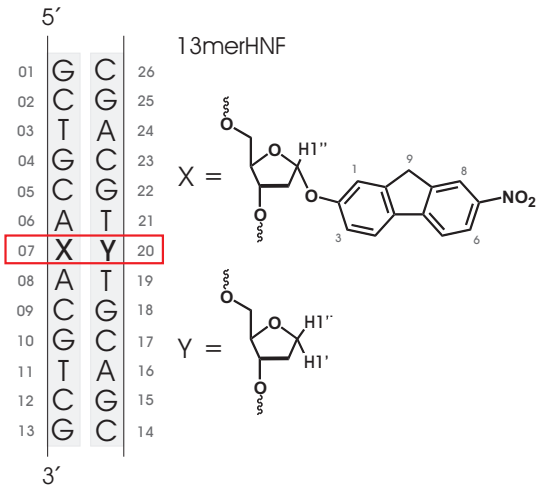


Fig. 4.17: *DNA duplex sequence with chemical structure of the HNF (**X**) and abasic site (**Y**).*

hand strand and **Y**=ABA (abasic site) in the other one (cf. Fig. 4.17). A symmetric, nonpalindromic 13 base pair sequence was chosen to dismiss the possibility of mispairing, loop formation and fraying effects. As in 13mer2AP, the central base pair was chosen for modification, in order to avoid fraying effects. The structures and nomenclature of HNF and the abasic site are also depicted in Fig. 4.17. For clarity only hydrogens which are important for the definition of the structure are shown. The nomenclature of the sugar protons follows the one described in section 2.1, with the hydrogen in the β-position of C1' symbolized by H1". Grey numbers indicate the numbering scheme of the carbon atoms and the corresponding hydrogens of the fluorene body (Fig. 4.17).

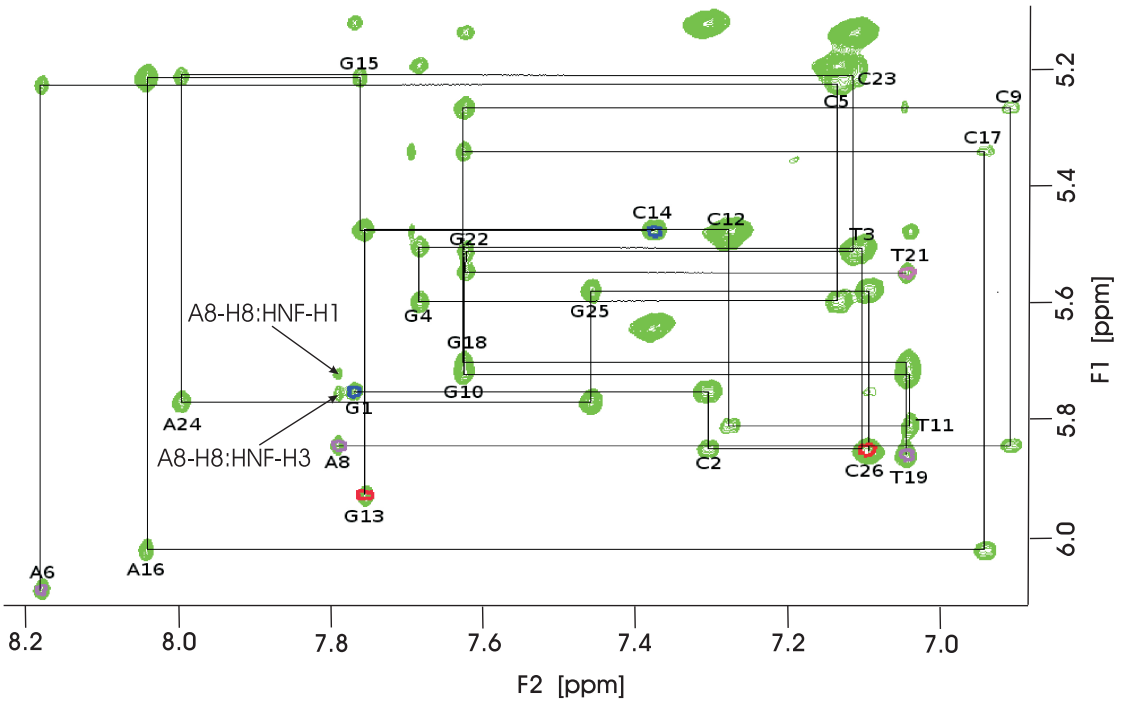


Fig. 4.18: *Sequential assignment in the sugar H1' base H6/H8 region of the NOESY-spectrum in D_2O for 13merHNF. Only the intraresidual cross-peaks H1'-H6/H8 are marked for clarity. Starting points are marked with blue, end points with red circles. Termination points of the sequential assignment within one strand due to the modification site are marked with purple circles.*

4.2.2 Results

4.2.2.1 Chemical shift analysis

The assignment of the DNA signals was achieved following standard procedures as described in Roberts [1993] and Bloomfield et al. [2000]. In contrast to 13mer2AP however, the sequential assignment was interrupted at the modification site due to the lack of base protons at the abasic site (cf. Fig. 4.18, indicated by purple circles). Assignment at the modification site was however possible through several inter- and intrastrand NOE cross-peaks from the HNF H5, H6, H8 and H1, H3, H4 protons to residues T19, ABA20, T21 and A6, A8, respectively. As an example, the NOE cross-peaks A8-H8:HNF7-H1 and A8-H8:HNF7-H3 are marked in Fig. 4.18.

Based on the assignment of the H1' and H6/H8 protons, the remaining base and sugar protons (including HNF and ABA) could be assigned by combining the information from

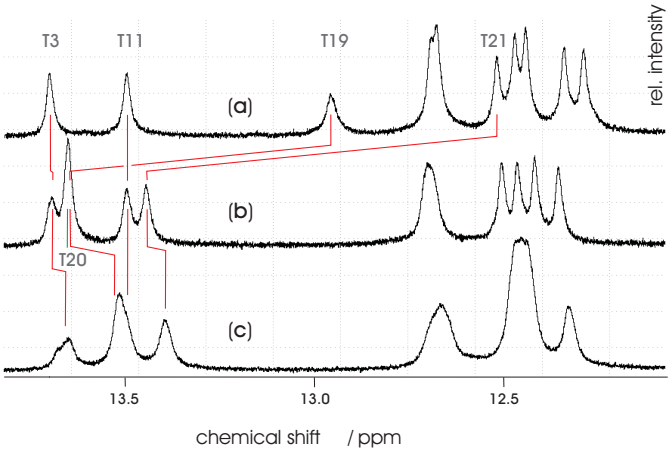


Fig. 4.19: *Comparison of the imino proton region of 13merRef, 13merRefGC and 13merHNF. Red solid lines follow the chemical shift assignments of a given imino proton resonance starting from 13merHNF (a) through 13merRef (b) to 13merRefGC (c). The solid green line indicates the chemical shift of the T20 imino proton which does not exist in the 13merHNF duplex.*

the COSY-, TOCSY-, HMQC-, and NOESY-spectra. Assignment of the exchangeable protons was done in the WATERGATE-NOESY-spectrum. Because of the symmetry of the sequence the imino, amino, and H2 protons had to be referenced to the already assigned non-exchangeable H5 protons via the H42/H41-H5 NOE cross-peaks. Assignment of the imino protons of T19 and T21 was complicated by the strong upfield shift of both protons when compared to either corresponding protons in 13merRef and 13merRefGC (see Fig. 4.19).

CSD analysis of 13merHNF and the corresponding native structures 13merRef (with a central A:T base pair) and 13merRefGC (with a central G:C base pair) is shown in Fig. 4.20, red and blue columns respectively. For comparison the CSDs between the to native structures 13merRef and 13merRefGC are shown (green columns). The sum of the absolute values of the CSDs for all protons belonging to one residue is given in order to avoid cancelling of negative and positive shifts. This results in a loss of information regarding the direction of the chemical shift differences, but allows for comparison between the different residues. With the exception of the three central base pairs, absolute per-residue CSDs upon introduction of HNF are comparable or only slightly larger than for the exchange of an A:T vs a G:C base pair (Fig. 4.20). Residues that are more

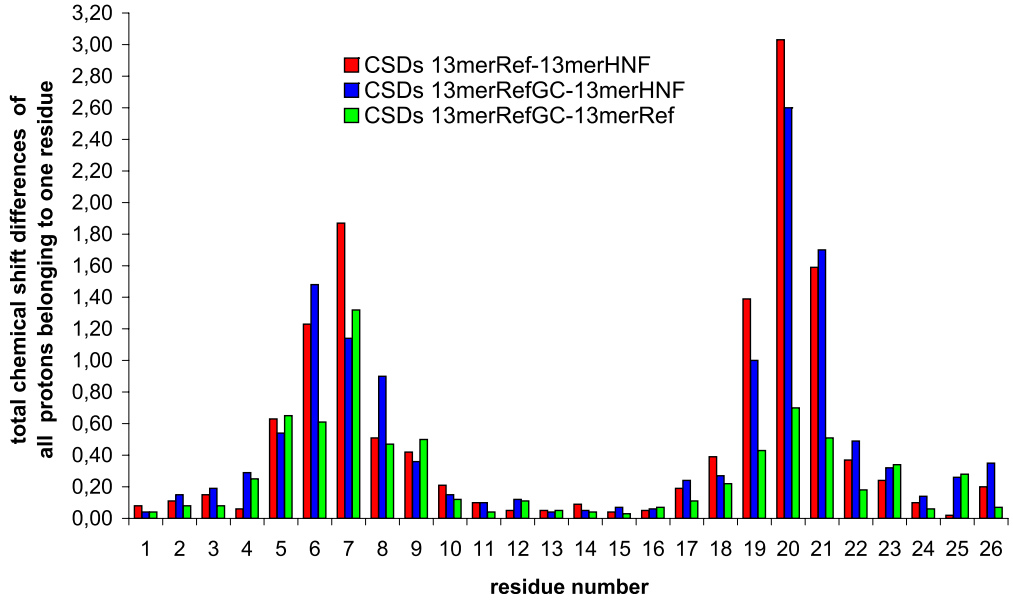


Fig. 4.20: *The sum of the absolute values of the CSDs for all protons belonging to one residue is given for: 13merRef to 13merRefGC (green columns), 13merHNF to 13merRef (red columns) and 13merHNF to 13merRefGC (blue columns). 13merRefGC has the same sequence as 13merRef but with a central G:C base pair.*

than two base pairs removed from the modification site do not exhibit significant chemical shift perturbations. The chemical shifts of all assigned proton and carbon atoms for 13merHNF, 13merRefGC and 13merRef are given in the Appendix, sections .6, .7 and .8.

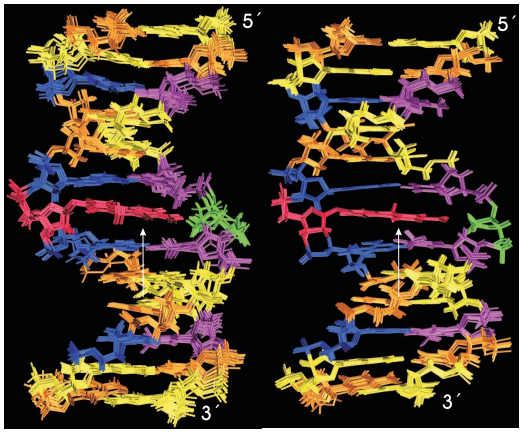


Fig. 4.21: *The 10 minimum-energy, violation-free structures of 13merHNF in face-up (left panel) and face-down (right panel) orientation are depicted. White arrows indicate the view direction of Fig. 4.23*

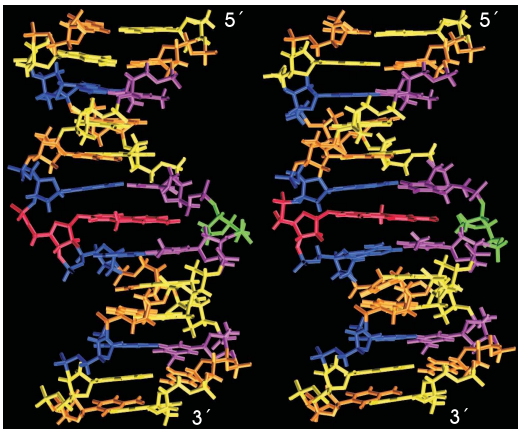


Fig. 4.22: *The averaged, minimized structures of 13merHNF in face-up (left panel) and face-down (right panel) orientation are depicted.*

4.2.2.2 NMR solution structure

The NMR solution structure is determined from experimental NOE and RDC data as described in section 3.1.4. While we see only one NOE data set for the duplex as a whole, the subset relating to the HNF chromophore cannot be described by a single orientation. Instead two structures of the same duplex are needed with different orientations of the chromophore: one where the fluorene methylene group points towards the major groove (face-up) and one where it points to the minor groove (face-down). Interestingly, the RDC restraints allow both orientations equally well. Simulated Annealing calculations for the two orientations produced two families of structures. The best (minimum-energy, violation-free) 10 of each, which are shown in Fig. 4.21, are used for generating the average structures. The latter are depicted in Fig. 4.22. The HNF probe fits into the helical fold (Fig. 4.21 and 4.22) and stacks with residues 6, 8, 21 and partly 19. This is illustrated in Fig. 4.23, where the central three base pairs of each orientation are zoomed and rotated to visualize stacking interactions.

The calculation results were validated by back-calculation of the NOESY-spectra (red and blue for face-up and face-down orientations respectively) and comparison with the experimental one (green). Fig. 4.24a and 4.24b differ only in the displayed region. While

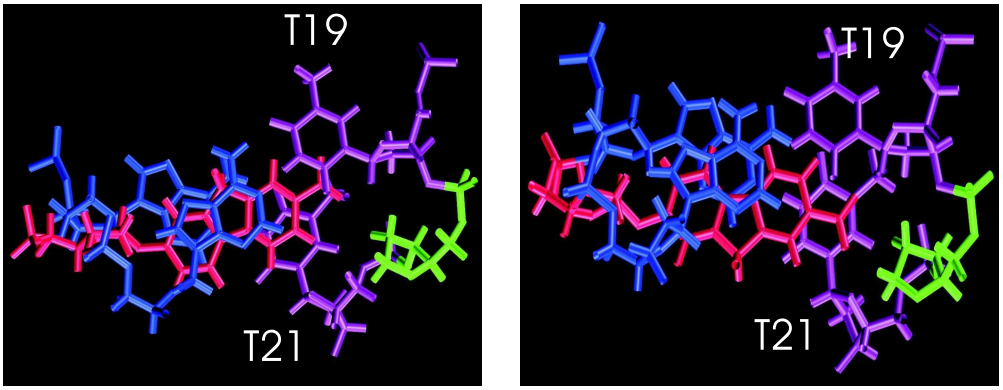
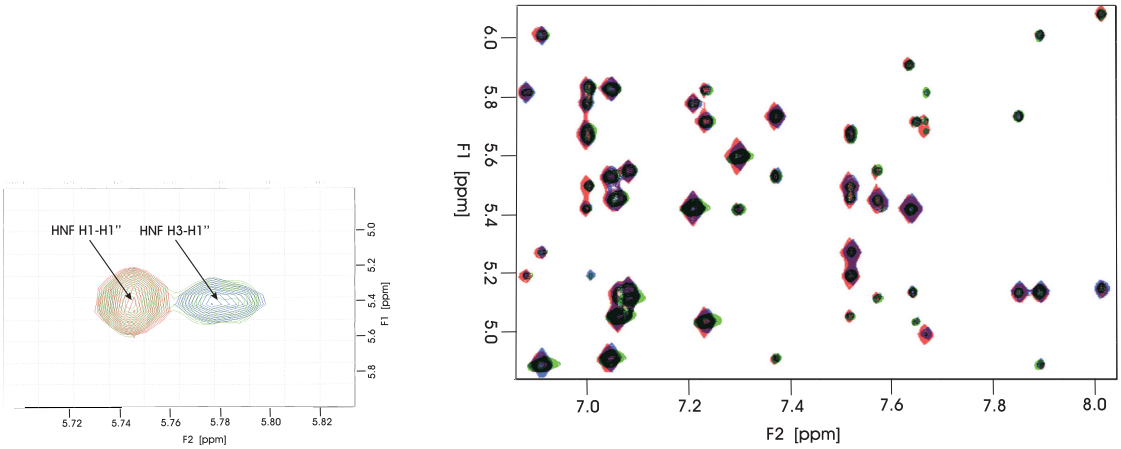
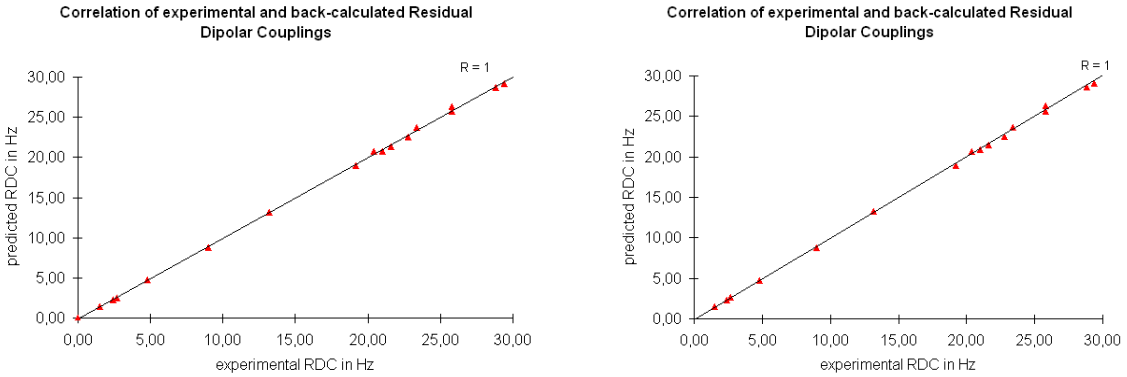


Fig. 4.23: *The central three base pairs of the average structures of 13merHNF in face-up (left panel) and face-down (right panel) orientation are depicted.*



(a) *H1/H3-H1''* **(b)** *H6/H8-H1'*

Fig. 4.24: *Overlay of the experimental NOESY-spectrum at 150 ms mixing time (green) and the NOESY-spectra back-calculated from the averaged, minimized structures of the HNF in the face-up (blue) and face-down (red) orientation. Arrows point to NOE cross-peaks involving the modification site. Panel (a) depicts the H1'-H1/H3 NOE cross-peaks, which can be only accounted for with the two different orientations of HNF within the double helix. Panel (b) shows the H1'-H6/H8 region, where predicted spectra for both orientations fit the experimental one equally well.*



(a) *face-up* **(b)** *face-down*

Fig. 4.25: *Plot of the experimental vs predicted RDCs for 13merHNF with HNF in the face-up (a) and face-down (b) orientations respectively.*

the latter shows the H1'-H6/H8 proton region (the same as in Fig. 4.18), Fig. 4.24a centers on two characteristic intraresidual NOE cross-peaks, namely H1/H3-H1" of the HNF residue. The latter peaks can only be accounted for by the two different orientations of the HNF inside the double helix, as is demonstrated by the back-calculated spectra. As either peak would be non-observable with only one orientation sampled, the population ratio of the face-up and face-down orientation can be estimated from the integral ratio of these two peaks to be 1:1. The accuracy of the calculations has been further validated by back-calculation of the RDCs from the average structure. The correlation plots of experimentally determined vs predicted RDCs are shown in Fig. 4.25a (4.25b) and yielded correlation factors (R) of 1.000 and q-factors of 0.004 (0.005) for the face-up (face-down) orientations. The precision of the calculations is assessed by the RMSDs of 10 minimum-energy, violation-free structures to their average structure, which are 0.66 Å and 0.47 Å for the face-up and face-down structures, respectively. An analysis of the helical parameters of the duplex structure was not possible since the modification of the central base pair made the definition of the helical axis impossible for the commonly used programs 3DNA [Lu and Olson, 2003] and CURVES+ [Lavery et al., 2009].

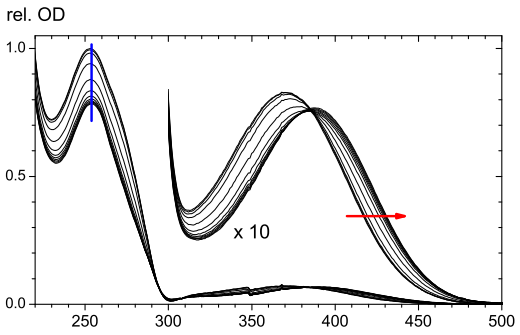


Fig. 4.26: *Absorption changes upon hybridisation, when lowering temperature from 85 to 25° C. The well-known hyperchromism of the UV absorbance (blue arrow) is accompanied by a 1190 cm^{-1} red-shift of the HNF absorption band.*

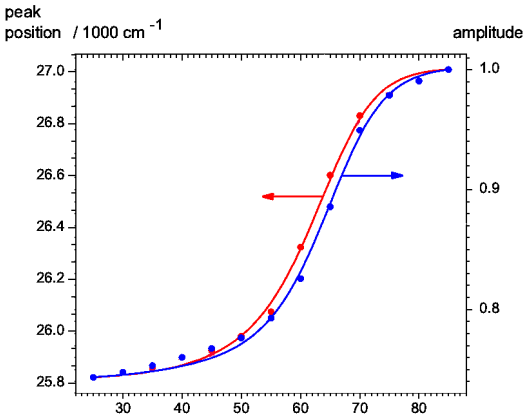


Fig. 4.27: *Temperature-dependence of the spectra in Fig. 4.26. The amplitude of the absorption peak around 260 nm (blue points, right scale) yields a melting point of 64 °C. The peak position of the HNF absorption band (red points, left scale) shows a melting point which is lower by 3 °C.*

4.2.2.3 Duplex melting

UV/vis absorption spectra of the DNA-HNF duplex are shown in Fig. 4.26 as a function of temperature. The nucleobases absorb intensely at 260 nm while the HNF chromophor is seen by a weak band around 380 nm, corresponding to the S1→S0 transition. At 85 °C only single strands are present. As the temperature is lowered to 25 °C, the HNF absorption band experiences a red-shift while the nucleobase absorption decreases.

Spectral change with temperature is quantified by "melting curvesäs shown in Fig. 4.27. The relative UV absorption amplitude decreases from 1 in the single strands to 0.74 in the duplex (right scale, blue points). A melting point T_m of 64 °C is found for the total concentration c_T=23.5 mM of the single strands. The HNF peak position is shown as red points (left scale) in Fig. 4.27. With this measure the melting point is located 3 K lower.

4.2.3 Discussion

Orientational flexibility of the HNF residue is indicated by the existence of two equally strong NOE cross-peaks between the H1" of the sugar and the H1 and H3 protons, respectively (cf. Fig. 4.24a). From their integrals a 1:1 population ratio could be estimated. Another possible explanation for the observation of a 1:1 integral ratio for these peaks might be spin diffusion effects. This possibility can be ruled out, as the integral ratio exhibited no dependence on the NOESY mixing time parameter. Calculations on both orientations and subsequent spectrum prediction support this conclusion. Interchange between the two orientations of HNF involves a 180 ° flip around the C1'-O2-bond which links the HNF to the sugar (cf. Fig. 4.17 and 4.22). This can take place only during transient opening of the formal HNF-abasic site base pair. For natural base pairs such opening motions are observed on a millisecond timescale . Another argument for orientational flip on that timescale is that NOE cross-peak signals would appear as an average of both orientations. This might explain why only one NOE data set is observed with some NOE cross-peaks involving the HNF chromophore being exclusively consistent with either orientation and others which can be used to describe both orientations but only with increased error bounds. The consistency of the RDC data with either orientation can be explained by the orientational degeneracy of the small experimental RDC set measured in a single orientational medium (cf. section 2.3.3). Thus one can conclude that the HNF chromophore undergoes orientational flip on a ms timescale.

Possible causes for the orientational degeneracy might be the introduction of the abasic site. The latter has already been reported in the literature to severely increase the flexibility of the helical structure in the vicinity [Coppel et al., 1997, Lin et al., 1998, Hoehn et al., 2001, Lin and de los Santos, 2001, Smirnov et al., 2002, Chen et al., 2007, 2008]. Another reason might be that the HNF chromophore is a conjugated π-system which is largely devoid of functional groups (with the exception of the hydroxyl and the nitro groups at position 2 and 7, respectively). The charge distribution obtained by the DFT calculations described in section 3.1.3 is roughly symmetrical to a 180 °-flip around the long HNF axis. Thus no preference in terms of electronic interaction with

the adjacent base pairs can occur.

In both orientations the HNF chromophore intercalates into the DNA double helix despite its α-glycosidic attachment to the sugar (instead of β for natural nucleotides). This is indicated by the existence of interstrand NOE cross-peaks from the T19, ABA20 and T21 residues to the H5, H6, and H8 protons of HNF as well as the intrastrand ones from the A6 and A8 residues to the H1, H3 and H4 protons. The comparison of the CSD data for 13merHNF, 13merRef and 13merRefGC shows that significant CSDs occur exclusively for the central five base pairs. This suggests that by HNF incorporation the helical structure of the DNA is mainly perturbed two base pairs in each direction while the overall B-DNA conformation remains intact.

Stacking interactions with residues 6, 8, 21 and partly 19 are illustrated in Fig. 4.23. While T21 is fully stacked with the HNF moiety, the T19 residue is turned outward, away from HNF. Thus stacking interactions do not stabilize the T19:A8 base pair as well as the other base pairs. As a consequence the T19 imino proton is shifted up-field and broadened, similar to what is observed with semi-terminal imino protons [Nonin et al., 1995]. This is illustrated by the NMR spectra in Fig. 4.19 where the imino protons of the HNF-substituted duplex (a) are compared to those of the same duplex containing a central A:T (b) or G:C (c) base pair. The T21 imino proton on the other hand, though also shifted upwards significantly, exhibits a very sharp resonance due to the ring current induced by HNF and the stabilizing effect of strong stacking interactions with the latter.

The thermodynamics of duplex formation for 13merHNF has been examined, to probe whether HNF intercalates and how stable the local and global helical arrangement is. When monitored via the temperature-dependent 260 nm absorbance of the nucleobases, a melting point of 64.0 °C is found. A comparison with the melting point of 13merRefGC is instructive. Under the same conditions, it is determined to be T_m=69.35 °C. From a comparison of standard hybridization enthalpies and with the assumption of a common hybridization entropy of -1.2 kJ/mol [Xia et al., 1998], 13merHNF is found to be less stable by 6.9 kJ/mol compared to 13merRefGC. This is equivalent to the lack of enthalpy from hydrogen bonding. When following the red-shift of the absorption band of HNF

at 380 nm, the melting point is estimated at 61.0 °C. The difference in melting points of 3.5 K can be compared to the 0.5 K which are found for the 13mer2AP duplex (see section 4.1.2.4). The large difference found for 13merHNF indicates considerable premelting around the HNF chromophore. This is in line with the results from the structure calculations and chemical shift analysis, which show orientational exchange and less favorable stacking interactions of HNF with T19.

In the past, base pair mimics devoid of hydrogen bonding have been demonstrated to be incorporated by DNA polymerases with comparable efficiency and even higher selectivity than natural bases due to steric complementarity [Morales and Kool, 1998, Guckian et al., 1998, Matray and Kool, 1999, Guckian et al., 2000]. The pyrene nucleotide, for example, can sterically mimic a WC base pair and is incorporated into DNA duplexes opposite to an abasic site without disruption of structure or decrease in duplex stability [Matray and Kool, 1998, Singh et al., 2002, Smirnov et al., 2002]. But pyrene has a pronounced effect on the local dynamics of adjacent base pairs, indicated by the presence of two interconverting resonances for the thymine imino proton to the 5'-side and broadening of the imino proton of the adjacent G:C base pair [Smirnov et al., 2002]. The fact that for the HNF-containing DNA duplex we do not observe interconverting signals, indicates that the perturbation of local dynamics is weaker than for a pyrene residue. However, substantial broadening of the T19 imino proton and orientational flip of the HNF indicate that local flexibility is also induced.

Summary

Structural and dynamic perturbations in DNA upon incorporation of either fluorophore, 2-Aminopurine (2AP) or 2-Hydroxy-7-nitrofluorene (HNF), are characterized by NMR spectroscopy. For this purpose the NMR solution structures of the modified DNA duplexes with the sequence 5'-GCTGCAXACGTCG-3' are solved. For X=2AP (13mer2AP) the partner base in the complementary strand is T, while for X=HNF (13merHNF) an abasic site is introduced to avoid steric strain.

As a structural isomer of A, the fluorescence properties of 2AP are commonly utilized to monitor stacking-unstacking transitions in molecular biology. By comparing results on 13mer2AP with the corresponding unmodified DNA duplex (13merRef, X=A), any perturbation can be unambiguously assigned to 2AP incorporation. For the NMR solution structure of 13merRef and 13mer2AP small but significant changes in helical parameters are found throughout the helix. Imino proton exchange measurements reveal an extended, distributed effect of 2AP incorporation on the lifetimes of the central seven base pair. This effect is explained by decreased activation enthalpy for base pair opening due to weakened stacking interactions and hydrogen bonding. The latter are indicated by the reduced melting point of 13mer2AP compared to 13merRef. Local melting around the modification site, as sensed by 2AP fluorescence, further supports the results from base pair dynamics. However, the reduced base pair lifetime of 2AP:T cannot fully account for the rapid water exchange observed with saturation transfer experiments in the absence of base catalyst. This indicates enhanced intrinsic catalysis. As a possible catalytic site the T O4 atom opposite 2AP is discussed, which is easily accessible through the major groove and lacks a hydrogen bonding partner within the base pair.

HNF is a fluorene derivative which was designed as a molecular probe for THz vibrational activity in biomolecules. Due to its elongated shape it is introduced opposite to an abasic site, which is known to induce orientational flexibility into DNA helices. The overall NMR solution structure is found to be B-DNA. However the NOE cross-peaks involving the HNF residue can only be accounted for by two different orientations of the HNF inside the DNA helical stack. Their population ratio is estimated to be 1:1. Dynamical perturbation is indicated by the increased linewidth and strong upfield shift of the T residue to the 5'-side of the abasic site. This can be explained with the help of the solution structure, which shows that this T residue cannot stack efficiently with the HNF. Lack of one stacking partner leads to destabilization of the base pair, as is oberved for the helical termini.

The dynamic as well as the structural perturbation due to HNF incorporation is large compared to the perturbations induced upon 2AP incorporation. This is not surprising considering the rather artificial shape of HNF as compared to 2AP. Furthermore, 2AP incorporation does not require introduction of an abasic site, which causes orientational flexibility within the double helix. In conclusion, HNF is ill-suited for application to biological problems. Changes observed with 2AP are much smaller. Structural differences are weak but the extended, distributed effect of 2AP incorporation on base pair opening rates limits its value for biologically significant applications such as monitoring stacking-unstacking transitions.

Future development should concentrate on the design of a fluorophore with the demonstrated spectral properties of HNF [Dallmann et al., 2009] but the more native shape of 2AP. For example, the introduction of an electron withdrawing group at the 6-position of 2AP would be an interesting future goal. While a relevant structure (6-Cyano-2-Aminopurine) has already been designed [Hocek and Holý, 1995], attachment to the sugar and incorporation into a DNA sequence have not been demonstrated yet. The introduction of another functional group to 2AP at the 6-position would circumvent the problem of solvent attack and thus reduce the dynamic as well as thermodynamic destabilization.

Zusammenfassung

Mittels NMR-Spektroskopie werden Störungen in Struktur und Dynamik von DNA untersucht, die durch den Einbau jeweils eines der beiden Fluorophore 2-Aminopurin (2AP) und 2-Hydroxy-7-nitrofluoren (HNF) hervorgerufen werden. Zu diesem Zweck werden die NMR-Strukturen der modifizierten Duplexe mit der Sequenz 5'-GCTGCAXACGTCG-3' berechnet. Im Fall X=2AP (13mer2AP) ist die Partnerbase im Komplementärstrang ein T, während gegenüber X=HNF (13merHNF) eine abasische Stelle eingeführt wird.

Als Strukturisomer von A, werden die Fluoreszenzeigenschaften von 2AP gern genutzt, um stacking-unstacking Übergänge in der Molekularbiologie zu verfolgen. Durch den Vergleich der Ergebnisse zum 13mer2AP mit denjenigen des entsprechenden unmodifizierten DNA Doppelstranges (13merRef, X=A) konnte jegliche Änderung eindeutig dem Einbau von 2AP zugordnet werden. Für die NMR-Strukturen von 13merRef und 13mer2AP können kleine aber signifikante, über die gesamte Helix verteilte Strukturstörungen nachgewiesen werden. Experimente zum Iminoprotonenaustausch mit Wasser ergeben, daß der Einbau von 2AP die Basenpaarlebensdauern der 7 zentralen Basenpaare erniedrigt. Dieser ausgedehnte Effekt kann durch eine verminderte Aktivierungsenthalpie für die Öffnung des Basenpaares erklärt werden. Ursachen dafür können schwächere Stapelwechselwirkungen und Wasserstoffbrückenbindungen sein. Geringere Energiebeiträge aus letzteren Wechselwirkungen können aus dem niedrigeren Schmelzpunkt des 13mer2AP Duplex abgeschätzt werden. Die DNA Schmelzpunktsbestimmung mittels 2AP-Fluoreszenz deutet lokales Schmelzen um die Modifikationsstelle herum an, was die Ergebnisse zur Basenpaardynamik zusätzlich unterstützt. Die kürzere Lebensdauer des 2AP:T Basenpaares kann jedoch nicht den schnellen Wasseraustausch

im Sättigungstransfer-Experiment ohne Zugabe von Basenkatalysator erklären. Als Erklärung für diese Diskrepanz wird eine effizientere intrinsische Katalyse vermutet. Als mögliche, katalytisch aktive Stelle wird das T O4 Atom diskutiert, welches über die große Furche leicht zugänglich ist und das keine Wasserstoffbrückenbindung innerhalb des Basenpaares ausbilden kann.

HNF ist ein Fluorenderivat das als molekulare Sonde für vibratorische Aktivität von Biomolekülen im THz-Bereich entwickelt wurde. Aufgrund seiner langgestreckten Form, wird statt einer Partnerbase eine abasische Stelle eingesetzt. Von letzterer ist jedoch bekannt, daß sie die helikale Struktur lokal flexibilisiert. Die übergeordnete Struktur des 13merHNF ist eine B-Form DNA Helix. Die NOE Kreuzpeaks zu den Protonen im HNF können jedoch nur durch zwei verschiedene Orientierungen des HNFs in der helikalen Anordnung beschrieben werden. Das Verhältnis der beiden Orientierungen untereinander wird als 1:1 abgeschätzt. Störungen in der Basenpaardynamik werden durch die höhere Linienbreite und die starke Hochfeldverschiebung des T auf der 5'-Seite ausgehend von der abasischen Stelle angedeutet. Eine Erklärung hierfür kann mit Hilfe der berechneten Struktur gegeben werden. Diese zeigt, daß das T keine effektiven Basenstapelwechselwirkungen mit HNF eingehen kann. Das Fehlen eines Wechselwirkungspartners führt zur Destabilisierung des Basenpaares, was z.B. auch für Basenpaare an den Helixtermini beobachtet wird.

Sowohl die dynamischen als auch strukturellen Änderungen nach Einbau von HNF sind groß im Vergleich zu den Störungen die durch den Einbau von 2AP verursacht werden. Eine Erklärung ist die stärkere Abweichung von der Struktur natürlicher Nukleobasen von HNF im Vergleich zu 2AP. Außerdem wird beim Einbau von 2AP keine abasische Stelle im Komplementärstrang benötigt, welche die Flexibilität der DNA-Helix lokal erhöhen würde. Das Fazit ist, das HNF für Anwendungen im biologischen Bereich nicht geeignet ist. Die Änderungen beim Einbau von 2AP sind wesentlich kleiner. Die strukturellen Unterschiede sind schwach, aber der ausgedehnte Effekt des 2AP-Einbaus auf die Basenpaarlebensdauern schränkt die Einsatzmöglichkeiten für biologische Fragestellungen wie stacking-unstacking Übergänge ein.

Die zukünftige Entwicklung neuartiger Fluorophore sollte sich an den bereits demonstrierten spektralen Eigenschaften des HNF [Dallmann et al., 2009] und der deutlich natürlicheren Struktur des 2AP orientieren. Die Einführung einer elektronenziehenden Gruppe in der 6-Position des 2AP stellt ein attraktives Syntheseziel dar. Während eine relevante Struktur (6-Cyano-2-Aminopurine) eines entsprechenden Fluorophores bereits beschrieben wurde [Hocek and Holý, 1995], konnte die glycosidische Verknüpfung und der Einbau in DNA noch nicht realisiert werden. Durch das Einführen einer weiteren funktionellen Gruppe in der 6-Position des 2AP könnte sowohl die dynamische als auch die thermodynamische Destabilisierung verhindert werden.

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Appendices

Script code

.1 Force field parameter and topology files

In this section the force field parameter and topology files, respectively, that were used throughout the calculations are presented. Changes or additions that were introduced by the author are marked as “mod by anda”.

```
   language
!RNA PARAMETER FILE  'FRAMEOWRK' FROM PARALLHDG.DNA AND ATOM NAMES
! AND HEAVY ATOM PARAMETERS FROM DNA-RNA.PARAM
!INCLUDES ALL NONEXCHANGEABLE HYDROGEN TERMS FOR BOND, ANGLE, AND
!IMPROPERS WITH ENERGY CONSTANT VARIABLES: $kchbond, $kchangle, AND $kchimpr.
!BOND, ANGLE, AND IMPROPERS WERE ESTIMATED FROM VALUES FROM THE STANDARD
!NUCLEOTIDES OF INSIGHTII 95.0 (BIOSYM/MOLECULAR SIMULATIONS).
!CREATED 2/24/96-- JASON P. RIFE AND PETER B. MOORE
! DNA-RNA-ALLATOM.PARAM

set echo=off message=off end


! checkversion 1.0


evaluate ($kchbond = 2000)
evaluate ($kchangle  = 1000)
evaluate ($kchimpr = 1000)


!******************* mod by anda - HNF******************

 BOND PX1  OX2     1489.209     1.485 ! Nobs =     1
 BOND PX1  OX3     1489.209     1.485 ! Nobs =     1
 BOND PX1  OX4     3350.720     1.593 ! Nobs =     1
 BOND OX4  CX5     1709.551     1.427 ! Nobs =     1
 BOND CX5  HX6     $kchbond     1.090 ! Nobs =     1
 BOND CX5  HX7     $kchbond     1.090 ! Nobs =     1
 BOND CX5  CX8     5235.500     1.511 ! Nobs =     1
 BOND CX8  HX9     $kchbond     1.090 ! Nobs =     1
 BOND CX8  OX10    2769.190     1.446 ! Nobs =     1
 BOND CX8  CX12    3350.720     1.528 ! Nobs =     1
 BOND OX10 CX11    1982.674     1.420 ! Nobs =     1
```

```
BOND CX11 CX14    1709.551     1.521 ! Nobs =     1
BOND CX11 OX18    1982.674     1.420 ! mod by anda, taken from C1'-O4' distance
BOND CX11 HX43    $kchbond     1.090 ! Nobs =     1
BOND CX12 HX13    $kchbond     1.090 ! Nobs =     1
BOND CX12 CX14    3350.720     1.518 ! Nobs =     1
BOND CX12 OX17    1982.674     1.431 ! Nobs =     1
BOND CX14 HX15    $kchbond     1.090 ! Nobs =     1
BOND CX14 HX16    $kchbond     1.090 ! Nobs =     1
BOND OX18 CX19    2769.190     1.446 ! mod by anda, taken from C4'-O4' distance
BOND CX19 CX20    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX19 CX24    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX20 CX21    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX20 HX36    $kchbond     1.090 ! Nobs =     1
BOND CX21 CX22    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX21 HX35    $kchbond     1.090 ! Nobs =     1
BOND CX22 CX23    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX22 CX25    1500.000     1.451 ! mod by anda, extrapolated from AGCT C5-C6 values
BOND CX23 CX24    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX23 CX27    1000.000     1.509 ! mod by anda, extrapolated from AGCT C5-C6 values
BOND CX24 HX40    $kchbond     1.090 ! Nobs =     1
BOND CX25 CX26    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX25 CX28    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX26 CX27    1500.000     1.509 ! mod by anda, extrapolated from AGCT C5-C6 values
BOND CX26 CX31    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX27 HX41    $kchbond     1.090 ! Nobs =     1
BOND CX27 HX42    $kchbond     1.090 ! Nobs =     1
BOND CX28 CX29    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX28 HX37    $kchbond     1.090 ! Nobs =     1
BOND CX29 CX30    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX29 HX38    $kchbond     1.090 ! Nobs =     1
BOND CX30 CX31    1500.000     1.391 ! mod by anda, extrapolated from AGCT C5-C6 values, mean
     distance from calc
BOND CX30 NX32    1370.370     1.471 ! mod by anda, k taken from C C4-N4, length from calc
BOND CX31 HX39    $kchbond     1.090 ! Nobs =     1
BOND NX32 OX33    1734.375     1.227 ! mod by anda, taken from T C2 ON
BOND NX32 OX34    1734.375     1.227 ! mod by anda, taken from T C2 ON

!****************** end mod by anda - HNF ****************

!the generic bonds were taken from param11.dna with 3*kq
BOND   C5R   OH        876.000     1.4300      ! 5' end
BOND   C5D   OH        876.000     1.4300      ! 5' end
BOND   C3R   OH        876.000     1.4300      ! 3' end
BOND   C3D   OH        876.000     1.4300      ! 3' end
BOND   O2R   HO       1350.000     0.9572
```

```
!Phos.  - combined RNA/DNA statistics used
!                        kq        x_eq        sigma
BOND  P     O1P      1489.209     1.485       ! 0.015  Phos
BOND  P     O2P      1489.209     1.485       ! 0.015  P
BOND  P     O5R      3350.720     1.593       ! 0.010  P
BOND  P     OH       3350.720     1.593       ! 0.010  P    ! For 5pho patch
BOND  P     O3R      2326.889     1.607       ! 0.012  P
BOND  P     OX17     2326.889     1.607       ! 0.012  P    ! mod by anda
BOND  PX1   O3R      2326.889     1.607       ! 0.012  P    ! mod by anda


!Sugars
!RNA statistics
BOND  O5R   C5R      1709.551     1.425       ! 0.014  Sugar
BOND  C5R   C4R      1982.674     1.510       ! 0.013  S
BOND  C4R   C3R      2769.190     1.524       ! 0.011  S
BOND  C3R   C2R      2769.190     1.525       ! 0.011  S
BOND  C2R   C1R      3350.720     1.528       ! 0.010  S
BOND  O4R   C1R      2326.888     1.414       ! 0.012  S
BOND  O4R   C4R      2326.888     1.453       ! 0.012  S
BOND  O3R   C3R      1982.674     1.423       ! 0.013  S
BOND  C2R   O2R      1982.674     1.413       ! 0.013  S

!DNA statistics
BOND  O5R   C5D      1709.551     1.427       ! 0.014  Sugar
BOND  C5D   C4D      5235.500     1.511       ! 0.008  S
BOND  C4D   C3D      3350.720     1.528       ! 0.010  S
BOND  C3D   C2D      3350.720     1.518       ! 0.010  S
BOND  C2D   C1D      1709.551     1.521       ! 0.014  S
BOND  O4D   C1D      1982.674     1.420       ! 0.013  S
BOND  O4D   C4D      2769.190     1.446       ! 0.011  S
BOND  O3R   C3D      1982.674     1.431       ! 0.013  S

!hydrogen/carbon
BOND  C4R   H        $kchbond    1.09
BOND  C3R   H        $kchbond    1.09
BOND  C2R   H        $kchbond    1.09
BOND  C1R   H        $kchbond    1.09
BOND  C5R   H        $kchbond    1.09

BOND  C4D   H        $kchbond    1.09
BOND  C3D   H        $kchbond    1.09
BOND  C2D   H        $kchbond    1.09
BOND  C1D   H        $kchbond    1.09
BOND  C5D   H        $kchbond    1.09

!Bases
!base specific bonds taken from param11.dna , 3*kq
BOND  O2U   HO       1350.000     0.957       ! UR
BOND  HN    NNA      1416.000     1.010       ! URA
BOND  HN    N1T      1416.000     1.010       ! Infer.
BOND  HN    N1C      1416.000     1.010
BOND  HN    N9G      1416.000     1.010
BOND  HN    N9A      1416.000     1.010
BOND  HN    N9P      1416.000     1.010
```

```
BOND  HN    N3U      1416.000    1.010
BOND  HN    N3T      1416.000    1.010
BOND  H2    N2       1416.000    1.010
BOND  H2    N4C      1416.000    1.010
BOND  H2    N2G      1416.000    1.010
BOND  H2    N6A      1416.000    1.010

BOND  HO    OH       1350.000    0.960      ! PARAM7 (IR stretch 3400 cm-1)

!Base sugar joint bonds (scale from sugar)
!                       kq        x_eq       sigma
BOND  C1R   N1T      1709.551    1.473      ! 0.014  Base
BOND  C1R   N1U      4136.691    1.469      ! 0.009  B
BOND  C1R   N1C      2326.889    1.470      ! 0.012  B
BOND  C1R   N9G      4136.691    1.459      ! 0.009  B
BOND  C1R   N9A      3350.720    1.462      ! 0.010  B
BOND  C1R   N9P      3350.720    1.462      ! 0.010  B

BOND  C1D   N1T      1709.551    1.473      ! 0.014  B   !DNA
BOND  C1D   N1U      4136.691    1.469      ! 0.009  B
BOND  C1D   N1C      2326.889    1.470      ! 0.012  B
BOND  C1D   N9G      4136.691    1.459      ! 0.009  B
BOND  C1D   N9A      3350.720    1.462      ! 0.010  B
BOND  C1D   N9P      3350.720    1.462      ! 0.010  B

!cytosine                        kq          x_eq  sigma
BOND        C2C     ON          1370.370    1.240 !0.009  B
BOND        C4C     N4C         1370.370    1.335 !0.009  B
BOND        N1C     C2C         1110.000    1.397 !0.010  B
BOND        N1C     C6C         3083.333    1.367 !0.006  B
BOND        C2C     NC          1734.375    1.353 !0.008  B
BOND        NC      C4C         2265.306    1.335 !0.007  B
BOND        C4C     C5C         1734.375    1.425 !0.008  B
BOND        C5C     C6C         1734.375    1.339 !0.008  B
BOND        C5C     H           $kchbond    1.09
BOND        C6C     H           $kchbond    1.09

!thymine
BOND        N1T     C2T         1734.375    1.376 !0.008  B
BOND        C2T     N3T         1734.375    1.373 !0.008  B
BOND        N3T     C4T         1734.375    1.382 !0.008  B
BOND        C4T     C5T         1370.370    1.445 !0.009  B
BOND        C5T     C6T         2265.306    1.339 !0.007  B
BOND        C6T     N1T         2265.306    1.378 !0.007  B
BOND        C2T     ON          1734.375    1.220 !0.008  B
BOND        C4T     ON          1370.370    1.228 !0.009  B
BOND        C5T     CC3E        3083.333    1.496 !0.006  B
BOND        C6T     H           $kchbond    1.09
BOND        CC3E    H           $kchbond    1.09

!adenine
BOND        NC      C2A         1370.370    1.339 !0.009  B
BOND        C2A     N3A         1370.370    1.331 !0.009  B
BOND        N3A     C4A         3083.333    1.344 !0.006  B
BOND        C4A     C5A         2265.306    1.383 !0.007  B
BOND        C5A     C6A         1370.370    1.406 !0.009  B
```

```
BOND        C6A     NC          2265.306    1.351 !0.007  B
BOND        C5A     N7A         3083.333    1.388 !0.006  B
BOND        N7A     C8A         2265.306    1.311 !0.007  B
BOND        C8A     N9A         1734.375    1.373 !0.008  B
BOND        N9A     C4A         3083.333    1.374 !0.006  B
BOND        C6A     N6A         1734.375    1.335 !0.008  B
BOND        C8A     H           $kchbond    1.08
BOND        C2A     H           $kchbond    1.09

!purine
BOND        NC      C2P         1370.370    1.339 !0.009  B
BOND        C2P     N3P         1370.370    1.331 !0.009  B
BOND        N3P     C4P         3083.333    1.344 !0.006  B
BOND        C4P     C5P         2265.306    1.383 !0.007  B
BOND        C5P     C6P         1370.370    1.406 !0.009  B
BOND        C6P     NC          2265.306    1.351 !0.007  B
BOND        C5P     N7P         3083.333    1.388 !0.006  B
BOND        N7P     C8P         2265.306    1.311 !0.007  B
BOND        C8P     N9P         1734.375    1.373 !0.008  B
BOND        N9P     C4P         3083.333    1.374 !0.006  B
BOND        C6P     H           $kchbond    1.09  !0.008  B
BOND        C8P     H           $kchbond    1.08
BOND        C2P     H           $kchbond    1.09

!guanine
BOND        NNA     C2G         1734.375    1.373 !0.008  B
BOND        C2G     N3G         1734.375    1.323 !0.008  B
BOND        N3G     C4G         2265.306    1.350 !0.007  B
BOND        C4G     C5G         2265.306    1.379 !0.007  B
BOND        C5G     C6G         1110.000    1.419 !0.010  B
BOND        C6G     NNA         2265.306    1.391 !0.007  B
BOND        C5G     N7G         3083.333    1.388 !0.006  B
BOND        N7G     C8G         3083.333    1.305 !0.006  B
BOND        C8G     N9G         2265.306    1.374 !0.007  B
BOND        N9G     C4G         1734.375    1.375 !0.008  B
BOND        C2G     N2G         1110.000    1.341 !0.010  B
BOND        C6G     O6G         1370.370    1.237 !0.009  B
BOND        C8G     H           $kchbond    1.08

!uracil
BOND        C2U     ON          1370.370    1.219 !0.009  B
BOND        C4U     ON          1734.375    1.232 !0.008  B
BOND        N1U     C2U         1370.370    1.381 !0.009  B
BOND        N1U     C6U         1370.370    1.375 !0.009  B
BOND        C2U     N3U         2265.306    1.373 !0.007  B
BOND        N3U     C4U         1370.370    1.380 !0.009  B
BOND        C4U     C5U         1370.370    1.431 !0.009  B
BOND        C5U     C6U         1370.370    1.337 !0.009  B
BOND        C5U     H           $kchbond    1.09
BOND        C6U     H           $kchbond    1.09
BOND        C2D     NX29        2265.306    1.479 !check param, added for pyr

!****************** mod by anda - HNF ********************

 ANGLe OX2   PX1   OX3      1337.074   119.600 ! Nobs =     1
 ANGLe OX2   PX1   OX4       357.719   108.100 ! Nobs =     1
```

```
ANGLe OX3   PX1   OX4       412.677   108.300 ! Nobs =     1
ANGLe PX1   OX4   CX5      1175.163   120.900 ! Nobs =     1
ANGLe OX4   CX5   HX6      $kchangle      109.70 ! Nobs =     1
ANGLe OX4   CX5   HX7      $kchangle      109.70 ! Nobs =     1
ANGLe OX4   CX5   CX8      1534.906   110.200 ! Nobs =     1
ANGLe HX6   CX5   HX7      $kchangle      109.17 ! Nobs =     1
ANGLe HX6   CX5   CX8      $kchangle      109.17 ! Nobs =     1
ANGLe HX7   CX5   CX8      $kchangle      109.17 ! Nobs =     1
ANGLe CX5   CX8   HX9      $kchangle      107.78 ! Nobs =     1
ANGLe CX5   CX8   OX10      429.678   109.400 ! Nobs =     1
ANGLe CX5   CX8   CX12      488.878   114.700 ! Nobs =     1
ANGLe HX9   CX8   OX10     $kchangle      112.98 ! Nobs =     1
ANGLe HX9   CX8   CX12     $kchangle      106.91 ! Nobs =     1
ANGLe OX10  CX8   CX12     1099.976   105.600 ! Nobs =     1
ANGLe CX8   OX10  CX11      650.874   109.700 ! Nobs =     1
ANGLe OX10  CX11  CX14      909.071   106.100 ! Nobs =     1
ANGLe OX10  CX11  OX18      357.719   107.016 ! Nobs =     1
ANGLe OX10  CX11  HX43     $kchangle      107.95 ! Nobs =     1
ANGLe CX14  CX11  OX18      488.878   111.73 ! mod by anda, gaussian value, kforce appr. from
    G,C,A,T
ANGLe CX14  CX11  HX43     $kchangle      112.29 ! Nobs =     1
ANGLe OX18  CX11  HX43     $kchangle      108.56 ! mod by anda, gaussian value
ANGLe CX8   CX12  HX13     $kchangle      111.16 ! Nobs =     1
ANGLe CX8   CX12  CX14     1099.976   103.200 ! Nobs =     1
ANGLe CX8   CX12  OX17      621.574   110.300 ! Nobs =     1
ANGLe HX13  CX12  CX14     $kchangle      111.98 ! Nobs =     1
ANGLe HX13  CX12  OX17     $kchangle      109.34 ! Nobs =     1
ANGLe CX14  CX12  OX17      412.677   110.600 ! Nobs =     1
ANGLe CX11  CX14  CX12      650.874   102.700 ! Nobs =     1
ANGLe CX11  CX14  HX15     $kchangle      112.29 ! Nobs =     1
ANGLe CX11  CX14  HX16     $kchangle      112.29 ! Nobs =     1
ANGLe CX12  CX14  HX15     $kchangle      111.36 ! Nobs =     1
ANGLe CX12  CX14  HX16     $kchangle      111.36 ! Nobs =     1
ANGLe HX15  CX14  HX16     $kchangle      107.52 ! Nobs =     1
ANGLe CX11  OX18  CX19      650.874   120.224 ! mod by anda, gaussian value, kforce c1-O4-C4
ANGLe OX18  CX19  CX20      621.574   124.456 ! mod by anda, gaussian value, kforce C4-C3-O3
ANGLe OX18  CX19  CX24      621.574   115.284 ! mod by anda, gaussian value, kforce C4-C3-O3
ANGLe CX20  CX19  CX24     1457.566   120.259 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX19  CX20  CX21     1457.566   120.215 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX19  CX20  HX36     $kchangle      120.775 ! mod by anda, gaussian value
ANGLe CX21  CX20  HX36     $kchangle      119.007 ! mod by anda, gaussian value
ANGLe CX20  CX21  CX22     1457.566   119.689 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX20  CX21  HX35     $kchangle      119.297 ! mod by anda, gaussian value
ANGLe CX22  CX21  HX35     $kchangle      121.012 ! mod by anda, gaussian value
ANGLe CX21  CX22  CX23     1457.566   119.780 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX21  CX22  CX25     1457.566   131.505 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX23  CX22  CX25     1457.566   108.714 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX22  CX23  CX24     1457.566   120.959 ! mod by anda, mean gaussian value, kforce C,A,G
    C4-C5-C6
ANGLe CX22  CX23  CX27     1457.566   109.983 ! mod by anda, mean gaussian value, kforce C,A,G
```

```
      C4-C5-C6
 ANGLe CX24 CX23 CX27     1457.566   129.058 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX19 CX24 CX23     1457.566   119.094 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX19 CX24 HX40     $kchangle      118.578 ! mod by anda, gaussian value
 ANGLe CX23 CX24 HX40     $kchangle      122.328 ! mod by anda, gaussian value
 ANGLe CX22 CX25 CX26     1457.566   108.593 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX22 CX25 CX28     1457.566   131.102 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX26 CX25 CX28     1457.566   120.305 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX25 CX26 CX27     1457.566   110.022 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX25 CX26 CX31     1457.566   120.807 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX27 CX26 CX31     1457.566   129.171 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX23 CX27 CX26     1457.566   102.689 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX23 CX27 HX41     $kchangle      111.842 ! mod by anda, gaussian value
 ANGLe CX23 CX27 HX42     $kchangle      111.916 ! mod by anda, gaussian value
 ANGLe CX26 CX27 HX41     $kchangle      111.832 ! mod by anda, gaussian value
 ANGLe CX26 CX27 HX42     $kchangle      111.824 ! mod by anda, gaussian value
 ANGLe HX41 CX27 HX42     $kchangle      106.844 ! mod by anda, gaussian value
 ANGLe CX25 CX28 CX29     1457.566   119.272 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX25 CX28 HX37     $kchangle      121.017 ! mod by anda, gaussian value
 ANGLe CX29 CX28 HX37     $kchangle      119.711 ! mod by anda, gaussian value
 ANGLe CX28 CX29 CX30     1457.566   119.411 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX28 CX29 HX38     $kchangle      121.419 ! mod by anda, gaussian value
 ANGLe CX30 CX29 HX38     $kchangle      119.171 ! mod by anda, gaussian value
 ANGLe CX29 CX30 CX31     1457.566   122.394 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX29 CX30 NX32      569.362   118.868 ! mod by anda, mean gaussian value, kforce A
      C5-C6-N6
 ANGLe CX31 CX30 NX32      569.362   118.738 ! mod by anda, mean gaussian value, kforce A
      C5-C6-N6
 ANGLe CX26 CX31 CX30     1457.566   117.812 ! mod by anda, mean gaussian value, kforce C,A,G
      C4-C5-C6
 ANGLe CX26 CX31 HX39     $kchangle      122.587 ! mod by anda, gaussian value
 ANGLe CX30 CX31 HX39     $kchangle      119.602 ! mod by anda, gaussian value
 ANGLe CX30 NX32 OX33      210.000   117.818 ! mod by anda, mean gaussian value, kforce A C-N-H
      double
 ANGLe CX30 NX32 OX34      210.000   117.978 ! mod by anda, mean gaussian value, kforce A C-N-H
      double
 ANGLe OX33 NX32 OX34     1337.074   124.204 ! mod by anda, mean gaussian value, kforce O-P-O

!******************* end of mod by anda - HNF *************

!Phos.
!the ANGLe s were taken from param11.dna with 3*kq
ANGLe        HO      OH      C5R          139.500   107.300
ANGLe        HO      O5R     C5R          139.500   107.300
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```
ANGLe       HO      OH      C5D         139.500   107.300
ANGLe       HO      O5R     C5D         139.500   107.300
ANGLe       HO      O3R     P           139.500   107.300
ANGLe       HO      OH      P           139.500   107.300 ! For 5pho patch
ANGLe       HO      O2R     C2R         139.500   107.300
ANGLe       OH      P       O3R         144.300   102.600 !
ANGLe       OH      P       O5R         144.300   102.600 !
ANGLe       OH      P       O1P         296.700   108.230 !
ANGLe       OH      P       O2P         296.700   108.230 !
ANGLe       OH      C5R     C4R         210.000   112.000 !
ANGLe       OH      C5D     C4D         210.000   112.000 !
ANGLe       C4D     C3D     OH          139.500   111.000 !
ANGLe       C4R     C3R     OH          139.500   111.000 !
ANGLe       C2D     C3D     OH          139.500   111.000 !
ANGLe       C2R     C3R     OH          139.500   111.000 !
ANGLe       C3R     OH      HO          139.500   107.300 !
ANGLe       C3D     OH      HO          139.500   107.300 !

!Phos.  - combined RNA/DNA statistics used
!                                        kq       x_eq  sigma
ANGLe       O1P     P       O2P        1337.074  119.600 !1.5 P
ANGLe       O5R     P       O1P         357.719  108.100 !2.9 P
ANGLe       O5R     P       O2P         412.677  108.300 !2.7 P
ANGLe       O3R     P       O5R         833.356  104.000 !1.9 P
ANGLe       OX17    P       O5R         833.356  104.000 !1.9 P !mod by anda
ANGLe       O3R     PX1     OX4         833.356  104.000 !1.9 P !mod by anda

ANGLe       O2P     P       O3R         293.791  108.300 !3.2 P
ANGLe       O1P     P       O3R         293.791  107.400 !3.2 P
ANGLe       OX3     PX1     O3R         293.791  108.300 !3.2 P !mod by anda
ANGLe       OX2     PX1     O3R         293.791  107.400 !3.2 P !mod by anda
ANGLe       O2P     P       OX17        293.791  108.300 !3.2 P !mod by anda
ANGLe       O1P     P       OX17        293.791  107.400 !3.2 P !mod by anda

ANGLe       O5R     C5R     C4R        1534.906  110.200 !1.4 P
ANGLe       P       O5R     C5R        1175.163  120.900 !1.6 P
ANGLe       P       O3R     C3R        2089.178  119.700 !1.2 P

ANGLe       O5R     C5D     C4D        1534.906  110.200 !1.4 P !DNA
ANGLe       P       O5R     C5D        1175.163  120.900 !1.6 P
ANGLe       P       O3R     C3D        2089.178  119.700 !1.2 P
ANGLe       PX1     O3R     C3D        2089.178  119.700 !1.2 P !mod by anda
ANGLe       P       OX17    CX12       2089.178  119.700 !1.2 P !mod by anda


!Sugars
!RNA statistics
!                                        kq       x_eq  sigma
ANGLe       O4R     C4R     C3R         561.212  105.500 !1.4 S
ANGLe       C5R     C4R     C3R         488.878  115.500 !1.5 S
ANGLe       C5R     C4R     O4R         561.212  109.200 !1.4 S
ANGLe       C1R     O4R     C4R        1357.996  109.600 !0.9 S
ANGLe       C4R     C3R     C2R        1099.976  102.700 !1.0 S
ANGLe       C3R     C2R     C1R        1357.996  101.500 !0.9 S
ANGLe       O4R     C1R     C2R         561.212  106.400 !1.4 S
ANGLe       N1T     C1R     C2R         429.678  113.400 !1.6 S
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```
ANGLe      N1C    C1R    C2R       429.678  113.400 !1.6 S
ANGLe      N1U    C1R    C2R       429.678  113.400 !1.6 S
ANGLe      N9G    C1R    C2R       429.678  113.400 !1.6 S
ANGLe      N9A    C1R    C2R       429.678  113.400 !1.6 S
ANGLe      N9P    C1R    C2R       429.678  113.400 !1.6 S
ANGLe      O4R    C1R    N1T      1099.976  108.200 !1.0 S
ANGLe      O4R    C1R    N1C      1099.976  108.200 !1.0 S
ANGLe      O4R    C1R    N1U      1099.976  108.200 !1.0 S
ANGLe      O4R    C1R    N9A      1099.976  108.200 !1.0 S
ANGLe      O4R    C1R    N9P      1099.976  108.200 !1.0 S
ANGLe      O4R    C1R    N9G      1099.976  108.200 !1.0 S
ANGLe      C1R    C2R    O2R       357.719  110.600 !2.9 S scale from phos.
ANGLe      C3R    C2R    O2R       357.719  113.300 !2.9 S scale from phos.
ANGLe      C4R    C3R    O3R       445.032  110.500 !2.6 S scale from phos.
ANGLe      C2R    C3R    O3R       383.726  111.000 !2.8 S scale from phos.

!DNA statistics
ANGLe      O4D    C4D    C3D      1099.976  105.600 !1.0 S
ANGLe      C5D    C4D    C3D       488.878  114.700 !1.5 S
ANGLe      C5D    C4D    O4D       429.678  109.400 !1.6 S
ANGLe      C1D    O4D    C4D       650.874  109.700 !1.3 S
ANGLe      C4D    C3D    C2D      1099.976  103.200 !1.0 S
ANGLe      C3D    C2D    C1D       650.874  102.700 !1.3 S
ANGLe      O4D    C1D    C2D       909.071  106.100 !1.1 S
ANGLe      N1T    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      N1C    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      N1U    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      N9G    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      N9A    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      N9P    C1D    C2D       488.878  114.200 !1.5 S
ANGLe      O4D    C1D    N1T      1357.996  107.800 !0.9 S
ANGLe      O4D    C1D    N1C      1357.996  107.800 !0.9 S
ANGLe      O4D    C1D    N1U      1357.996  107.800 !0.9 S
ANGLe      O4D    C1D    N9A      1357.996  107.800 !0.9 S
ANGLe      O4D    C1D    N9P      1357.996  107.800 !0.9 S
ANGLe      O4D    C1D    N9G      1357.996  107.800 !0.9 S
ANGLe      C4D    C3D    O3R       621.574  110.300 !2.2 S scale from phos.
ANGLe      C2D    C3D    O3R       412.677  110.600 !2.7 S scale from phos.

!Ribose terms involving non-exchageables
ANGLe      OH     C5R    H        $kchangle     109.83
ANGLe      O5R    C5R    H        $kchangle     109.83
ANGLe      H      C5R    H        $kchangle     109.11
ANGLe      C4R    C5R    H        $kchangle     109.11
ANGLe      C5R    C4R    H        $kchangle     107.93
ANGLe      H      C4R    C3R      $kchangle     107.13
ANGLe      H      C4R    O4R      $kchangle     113.74
ANGLe      H      C3R    C4R      $kchangle     111.35
ANGLe      H      C3R    O3R      $kchangle     105.87
ANGLe      H      C3R    OH       $kchangle     105.87
ANGLe      H      C3R    C2R      $kchangle     112.27
ANGLe      H      C2R    C3R      $kchangle     111.41
ANGLe      H      C2R    O2R      $kchangle     113.07
ANGLe      H      C2R    C1R      $kchangle     112.38
ANGLe      H      C1R    C2R      $kchangle     111.95
ANGLe      H      C1R    N1C      $kchangle     107.70
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ANGLe      H       C1R     N1U       $kchangle     107.70
ANGLe      H       C1R     N1T       $kchangle     107.70
ANGLe      H       C1R     N9A       $kchangle     107.70
ANGLe      H       C1R     N9P       $kchangle     107.70
ANGLe      H       C1R     N9G       $kchangle     107.70
ANGLe      H       C1R     O4R       $kchangle     106.86

!Deoxyribose terms involving non-exchageables
!
ANGLe      OH      C5D     H         $kchangle     109.70
ANGLe      O5R     C5D     H         $kchangle     109.70
ANGLe      H       C5D     H         $kchangle     109.17
ANGLe      C4D     C5D     H         $kchangle     109.17
ANGLe      C5D     C4D     H         $kchangle     107.78
ANGLe      H       C4D     C3D       $kchangle     106.91
ANGLe      H       C4D     O4D       $kchangle     112.98
ANGLe      H       C3D     C4D       $kchangle     111.16
ANGLe      H       C3D     O3R       $kchangle     109.34
ANGLe      H       C3D     OH        $kchangle     109.34
ANGLe      H       C3D     C2D       $kchangle     111.98
ANGLe      H       C2D     C3D       $kchangle     111.36
ANGLe      H       C2D     H         $kchangle     107.52
ANGLe      H       C1D     H         $kchangle     107.52 !mod by anda
ANGLe      H       C2D     C1D       $kchangle     112.29
ANGLe      H       C1D     C2D       $kchangle     110.94
ANGLe      H       C1D     N1C       $kchangle     108.25
ANGLe      H       C1D     N1U       $kchangle     108.25
ANGLe      H       C1D     N1T       $kchangle     108.25
ANGLe      H       C1D     N9A       $kchangle     108.25
ANGLe      H       C1D     N9P       $kchangle     108.25
ANGLe      H       C1D     N9G       $kchangle     108.25
ANGLe      H       C1D     O4D       $kchangle     107.95

!Bases
!cytosine                             kq        x_eq    sigma
ANGLe      C6C     N1C     C2C      2277.447   120.300 !0.40 B
ANGLe      N1C     C2C     NC        743.656   119.200 !0.70 B
ANGLe      C2C     NC      C4C      1457.566   119.900 !0.50 B
ANGLe      NC      C4C     C5C      2277.447   121.900 !0.40 B
ANGLe      C4C     C5C     C6C      1457.566   117.400 !0.50 B
ANGLe      C5C     C6C     N1C      1457.566   121.000 !0.50 B
ANGLe      N1C     C2C     ON       1012.199   118.900 !0.60 B
ANGLe      NC      C2C     ON        743.656   121.900 !0.70 B
ANGLe      NC      C4C     N4C       743.656   118.000 !0.70 B
ANGLe      C5C     C4C     N4C       743.656   120.200 !0.70 B
ANGLe      C6C     N1C     C1R       763.873   120.800 !1.20 B scale from sugar
ANGLe      C2C     N1C     C1R       909.071   118.800 !1.10 B scale from sugar
ANGLe      C6C     N1C     C1D       763.873   120.800 !1.20 B !DNA
ANGLe      C2C     N1C     C1D       909.071   118.800 !1.10 B
ANGLe      C4C     N4C     H2        105.000   120.000 !from param11.dna, 3*keq
ANGLe      H2      N4C     H2        105.000   120.000
ANGLe      N1C     C6C     H        $kchangle   119.63
ANGLe      C5C     C6C     H        $kchangle   119.36
ANGLe      C4C     C5C     H        $kchangle   121.54
ANGLe      C6C     C5C     H        $kchangle   121.54
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```
!thymine                                kq          x_eq     sigma
ANGLe      C6T     N1T     C2T         1457.566    121.300  !0.50 B
ANGLe      N1T     C2T     N3T         1012.199    114.600  !0.60 B
ANGLe      C2T     N3T     C4T         1012.199    127.200  !0.60 B
ANGLe      N3T     C4T     C5T         1012.199    115.200  !0.60 B
ANGLe      C4T     C5T     C6T         1012.199    118.000  !0.60 B
ANGLe      C5T     C6T     N1T         1012.199    123.700  !0.60 B
ANGLe      N1T     C2T     ON           569.362    123.100  !0.80 B
ANGLe      N3T     C2T     ON          1012.199    122.300  !0.60 B
ANGLe      N3T     C4T     ON          1012.199    119.900  !0.60 B
ANGLe      C5T     C4T     ON           743.656    124.900  !0.70 B
ANGLe      C4T     C5T     CC3E        1012.199    119.000  !0.60 B
ANGLe      C6T     C5T     CC3E        1012.199    122.900  !0.60 B
ANGLe      C6T     N1T     C1R          488.878    120.400  !1.50 B scale from sugar
ANGLe      C2T     N1T     C1R          429.678    118.200  !1.60 B scale from sugar
ANGLe      C6T     N1T     C1D          488.878    120.400  !1.50 B !DNA
ANGLe      C2T     N1T     C1D          429.678    118.200  !1.60 B
ANGLe      C2T     N3T     HN           105.000    116.500   !from param11.dna, 3*keq
ANGLe      C4T     N3T     HN           105.000    116.500
ANGLe      C5T     CC3E    H          $kchangle     109.50
ANGLe      H       CC3E    H          $kchangle     109.44
ANGLe      N1T     C6T     H          $kchangle     119.52
ANGLe      C5T     C6T     H          $kchangle     119.52

!adenine                                kq          x_eq     sigma
ANGLe      C6A     NC      C2A         1012.199    118.600  !0.60 B
ANGLe      NC      C2A     N3A         1457.566    129.300  !0.50 B
ANGLe      C2A     N3A     C4A         1457.566    110.600  !0.50 B
ANGLe      N3A     C4A     C5A          743.656    126.800  !0.70 B
ANGLe      C4A     C5A     C6A         1457.566    117.000  !0.50 B
ANGLe      C5A     C6A     NC          1457.566    117.700  !0.50 B
ANGLe      C4A     C5A     N7A         1457.566    110.700  !0.50 B
ANGLe      C5A     N7A     C8A         1457.566    103.900  !0.50 B
ANGLe      N7A     C8A     N9A         1457.566    113.800  !0.50 B
ANGLe      C8A     N9A     C4A         2277.447    105.800  !0.40 B
ANGLe      N9A     C4A     C5A         2277.447    105.800  !0.40 B
ANGLe      N3A     C4A     N9A          569.362    127.400  !0.80 B
ANGLe      C6A     C5A     N7A          743.656    132.300  !0.70 B
ANGLe      NC      C6A     N6A         1012.199    118.600  !0.60 B
ANGLe      C5A     C6A     N6A          569.362    123.700  !0.80 B
ANGLe      C8A     N9A     C1R          339.499    127.700  !1.80 B scale from sugar
ANGLe      C4A     N9A     C1R          339.499    126.300  !1.80 B scale from sugar
ANGLe      C8A     N9A     C1D          339.499    127.700  !1.80 B !DNA
ANGLe      C4A     N9A     C1D          339.499    126.300  !1.80 B
ANGLe      C6A     N6A     H2           105.000    120.000   !from param11.dna, 3*keq
ANGLe      H2      N6A     H2           105.000    120.000
ANGLe      N7A     C8A     H          $kchangle     123.16
ANGLe      N9A     C8A     H          $kchangle     123.16
ANGLe      NC      C2A     H          $kchangle     115.54
ANGLe      N3A     C2A     H          $kchangle     115.54

!purine                                 kq          x_eq     sigma
ANGLe      C6P     NC      C2P         1012.199    118.600  !0.60 B
ANGLe      NC      C2P     N3P         1457.566    129.300  !0.50 B
ANGLe      C2P     N3P     C4P         1457.566    110.600  !0.50 B
ANGLe      N3P     C4P     C5P          743.656    126.800  !0.70 B
```

```
ANGLe       C4P     C5P     C6P        1457.566   117.000 !0.50 B
ANGLe       C5P     C6P     NC         1457.566   117.700 !0.50 B
ANGLe       C4P     C5P     N7P        1457.566   110.700 !0.50 B
ANGLe       C5P     N7P     C8P        1457.566   103.900 !0.50 B
ANGLe       N7P     C8P     N9P        1457.566   113.800 !0.50 B
ANGLe       C8P     N9P     C4P        2277.447   105.800 !0.40 B
ANGLe       N9P     C4P     C5P        2277.447   105.800 !0.40 B
ANGLe       N3P     C4P     N9P         569.362   127.400 !0.80 B
ANGLe       C6P     C5P     N7P         743.656   132.300 !0.70 B
ANGLe       NC      C6P     H          $kchangle  120.164 !0.60 B !modified by anda
ANGLe       C5P     C6P     H          $kchangle  120.164 !0.80 B !modified by anda
ANGLe       C8P     N9P     C1R         339.499   127.700 !1.80 B scale from sugar
ANGLe       C4P     N9P     C1R         339.499   126.300 !1.80 B scale from sugar
ANGLe       C8P     N9P     C1D         339.499   127.700 !1.80 B !DNA
ANGLe       C4P     N9P     C1D         339.499   126.300 !1.80 B
ANGLe       N7P     C8P     H          $kchangle   123.16
ANGLe       N9P     C8P     H          $kchangle   123.16
ANGLe       NC      C2P     H          $kchangle   115.54
ANGLe       N3P     C2P     H          $kchangle   115.54

!guanine                               kq          x_eq     sigma
ANGLe       C6G     NNA     C2G        1012.199   125.100 !0.60 B
ANGLe       NNA     C2G     N3G        1012.199   123.900 !0.60 B
ANGLe       C2G     N3G     C4G        1457.566   111.900 !0.50 B
ANGLe       N3G     C4G     C5G        1457.566   128.600 !0.50 B
ANGLe       C4G     C5G     C6G        1012.199   118.800 !0.60 B
ANGLe       C5G     C6G     NNA        1457.566   111.500 !0.50 B
ANGLe       C4G     C5G     N7G        2277.447   110.800 !0.40 B
ANGLe       C5G     N7G     C8G        1457.566   104.300 !0.50 B
ANGLe       N7G     C8G     N9G        1457.566   113.100 !0.50 B
ANGLe       C8G     N9G     C4G        2277.447   106.400 !0.40 B
ANGLe       N9G     C4G     C5G        2277.447   105.400 !0.40 B
ANGLe       N3G     C4G     N9G        1012.199   126.000 !0.60 B
ANGLe       C6G     C5G     N7G        1012.199   130.400 !0.60 B
ANGLe       NNA     C2G     N2G         449.866   116.20  !0.90 B
ANGLe       N3G     C2G     N2G         743.656   119.900 !0.70 B
ANGLe       NNA     C6G     O6G        1012.199   119.900 !0.60 B
ANGLe       C5G     C6G     O6G        1012.199   128.600 !0.60 B
ANGLe       C8G     N9G     C1R         650.874   127.000 !1.30 B scale from sugar
ANGLe       C4G     N9G     C1R         650.874   126.500 !1.30 B scale from sugar
ANGLe       C8G     N9G     C1D         650.874   127.000 !1.30 B !DNA
ANGLe       C4G     N9G     C1D         650.874   126.500 !1.30 B
ANGLe       C2G     N2G     H2          105.000   120.000  !from param11.dna, 3*keq
ANGLe       H2      N2G     H2          105.000   120.000
ANGLe       C2G     NNA     HN          105.000   119.300
ANGLe       C6G     NNA     HN          105.000   119.300
ANGLe       N7G     C8G     H          $kchangle   122.91
ANGLe       N9G     C8G     H          $kchangle   122.91

!uracile                               kq          x_eq     sigma
ANGLe       C6U     N1U     C2U        1012.199   121.000 !0.60 B
ANGLe       N1U     C2U     N3U        1012.199   114.900 !0.60 B
ANGLe       C2U     N3U     C4U        1012.199   127.000 !0.60 B
ANGLe       N3U     C4U     C5U        1012.199   114.600 !0.60 B
ANGLe       C4U     C5U     C6U        1012.199   119.700 !0.60 B
ANGLe       C5U     C6U     N1U        1457.566   122.700 !0.50 B
```

```
ANGLe      N1U    C2U    ON          743.656   122.800 !0.70 B
ANGLe      N3U    C2U    ON          743.656   122.200 !0.70 B
ANGLe      N3U    C4U    ON          743.656   119.400 !0.70 B
ANGLe      C5U    C4U    ON         1012.199   125.900 !0.60 B
ANGLe      C6U    N1U    C1R         561.212   121.200 !1.40 B
ANGLe      C2U    N1U    C1R         763.872   117.700 !1.20 B
ANGLe      C6U    N1U    C1D         561.212   121.200 !1.40 B !DNA
ANGLe      C2U    N1U    C1D         763.872   117.700 !1.20 B
ANGLe      C4U    ON     HO          105.000   120.000 !from param11.dna, 3*keq
ANGLe      C2U    N3U    HN          105.000   116.500
ANGLe      C4U    N3U    HN          105.000   116.500
ANGLe      N1U    C6U    H         $kchangle   119.38
ANGLe      C5U    C6U    H         $kchangle   119.38
ANGLe      C4U    C5U    H         $kchangle   119.56
ANGLe      C6U    C5U    H         $kchangle   119.56
ANGLe      C3D    C2D    NX29       1457.566    110.00 !check param, added fpr pyr
ANGLe      H      C2D    NX29        500.00     109.51 !check param, added fpr pyr


{
!******************* mod by anda - HNF ********************

 DIHEdral OX3   PX1   OX4   CX5      750.0 0    180.00 ! Nobs =    1 ... Value =   172.75
 DIHEdral PX1   OX4   CX5   HX6      750.0 0    -60.00 ! Nobs =    1 ... Value =   -63.48
 DIHEdral PX1   OX4   CX5   HX7      750.0 0     60.00 ! Nobs =    1 ... Value =    55.20
 DIHEdral PX1   OX4   CX5   CX8      750.0 0    180.00 ! Nobs =    1 ... Value =   176.08
 DIHEdral OX4   CX5   CX8   HX9      750.0 0    180.00 ! Nobs =    1 ... Value =   172.35
 DIHEdral OX4   CX5   CX8   OX10     750.0 0    -60.00 ! Nobs =    1 ... Value =   -69.24
 DIHEdral OX4   CX5   CX8   CX12     750.0 0     60.00 ! Nobs =    1 ... Value =    52.07
 DIHEdral HX6   CX5   CX8   HX9      750.0 0     60.00 ! Nobs =    1 ... Value =    51.91
 DIHEdral HX6   CX5   CX8   OX10     750.0 0    180.00 ! Nobs =    1 ... Value =   170.31
 DIHEdral HX6   CX5   CX8   CX12     750.0 0    -60.00 ! Nobs =    1 ... Value =   -68.38
 DIHEdral HX7   CX5   CX8   HX9      750.0 0    -60.00 ! Nobs =    1 ... Value =   -66.18
 DIHEdral HX7   CX5   CX8   OX10     750.0 0     60.00 ! Nobs =    1 ... Value =    52.23
 DIHEdral HX7   CX5   CX8   CX12     750.0 0    180.00 ! Nobs =    1 ... Value =   173.54
 DIHEdral CX5   CX8   CX12  CX14     750.0 0   -120.00 ! Nobs =    1 ... Value =  -129.37
 DIHEdral CX5   CX8   CX12  OX17     750.0 0    120.00 ! Nobs =    1 ... Value =   112.32
 DIHEdral HX9   CX8   CX12  CX14     750.0 0    120.00 ! Nobs =    1 ... Value =   110.44
 DIHEdral HX9   CX8   CX12  OX17     750.0 0      0.00 ! Nobs =    1 ... Value =    -7.87
 DIHEdral OX10  CX8   CX12  HX13     750.0 0    120.00 ! Nobs =    1 ... Value =   111.59
 DIHEdral OX10  CX8   CX12  CX14     750.0 0      0.00 ! Nobs =    1 ... Value =    -5.92
 DIHEdral OX10  CX8   CX12  OX17     750.0 0   -120.00 ! Nobs =    1 ... Value =  -124.23
 DIHEdral OX18  CX11  CX14  HX15     750.0 0     90.00 ! Nobs =    1 ... Value =    80.69
 DIHEdral OX10  CX11  OX18  CX19     750.0 0    180.00 ! Nobs =    1 ... Value =   177.32
 DIHEdral CX14  CX11  OX18  CX19     750.0 0    -60.00 ! Nobs =    1 ... Value =   -67.60
 DIHEdral HX43  CX11  OX18  CX19     750.0 0     60.00 ! Nobs =    1 ... Value =    56.28
 DIHEdral CX8   CX12  CX14  HX16     750.0 0    -90.00 ! Nobs =    1 ... Value =   -88.75
 DIHEdral HX13  CX12  CX14  CX11     750.0 0    -90.00 ! Nobs =    1 ... Value =   -90.38
 DIHEdral OX17  CX12  CX14  HX15     750.0 0    -90.00 ! Nobs =    1 ... Value =   -93.06}
 DIHEdral OX18  CX19  CX20  CX21   24000.0 0    180.00 ! Nobs =    1 ... Value =  -179.61
 DIHEdral OX18  CX19  CX20  HX36   24000.0 0      0.00 ! Nobs =    1 ... Value =    -0.12
 DIHEdral CX24  CX19  CX20  CX21    2400.0 0      0.00 ! Nobs =    1 ... Value =    -0.46
 DIHEdral CX24  CX19  CX20  HX36    2400.0 0    180.00 ! Nobs =    1 ... Value =   179.03
 DIHEdral OX18  CX19  CX24  CX23   24000.0 0    180.00 ! Nobs =    1 ... Value =   179.62
 DIHEdral OX18  CX19  CX24  HX40   24000.0 0      0.00 ! Nobs =    1 ... Value =    -0.53
 DIHEdral CX20  CX19  CX24  CX23    2400.0 0      0.00 ! Nobs =    1 ... Value =     0.42
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DIHEdral CX20 CX19 CX24 HX40    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.73
DIHEdral CX19 CX20 CX21 CX22    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.26
DIHEdral CX19 CX20 CX21 HX35    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.84
DIHEdral HX36 CX20 CX21 CX22    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.24
DIHEdral HX36 CX20 CX21 HX35    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.67
DIHEdral CX20 CX21 CX22 CX23    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.02
DIHEdral CX20 CX21 CX22 CX25    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.94
DIHEdral HX35 CX21 CX22 CX23    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.92
DIHEdral HX35 CX21 CX22 CX25    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.03
DIHEdral CX21 CX22 CX23 CX24    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.02
DIHEdral CX21 CX22 CX23 CX27    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.89
DIHEdral CX25 CX22 CX23 CX24    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.98
DIHEdral CX25 CX22 CX23 CX27    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.07
DIHEdral CX21 CX22 CX25 CX26    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.90
DIHEdral CX21 CX22 CX25 CX28    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.02
DIHEdral CX23 CX22 CX25 CX26    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.06
DIHEdral CX23 CX22 CX25 CX28    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.98
DIHEdral CX22 CX23 CX24 CX19    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.18
DIHEdral CX22 CX23 CX24 HX40    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.97
DIHEdral CX27 CX23 CX24 CX19    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.93
DIHEdral CX27 CX23 CX24 HX40    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.08
DIHEdral CX22 CX23 CX27 CX26    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.05
DIHEdral CX22 CX23 CX27 HX41    2400.0 0    120.00 ! Nobs =     1 ... Value =    117.87
DIHEdral CX22 CX23 CX27 HX42    2400.0 0   -120.00 ! Nobs =     1 ... Value =   -117.81
DIHEdral CX24 CX23 CX27 CX26    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.95
DIHEdral CX24 CX23 CX27 HX41    2400.0 0    -60.00 ! Nobs =     1 ... Value =    -62.23
DIHEdral CX24 CX23 CX27 HX42    2400.0 0     60.00 ! Nobs =     1 ... Value =     62.09
DIHEdral CX22 CX25 CX26 CX27    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.03
DIHEdral CX22 CX25 CX26 CX31    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.97
DIHEdral CX28 CX25 CX26 CX27    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.96
DIHEdral CX28 CX25 CX26 CX31    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.04
DIHEdral CX22 CX25 CX28 CX29    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.92
DIHEdral CX22 CX25 CX28 HX37    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.06
DIHEdral CX26 CX25 CX28 CX29    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.01
DIHEdral CX26 CX25 CX28 HX37    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.97
DIHEdral CX25 CX26 CX27 CX23    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.01
DIHEdral CX25 CX26 CX27 HX41    2400.0 0   -120.00 ! Nobs =     1 ... Value =   -117.79
DIHEdral CX25 CX26 CX27 HX42    2400.0 0    120.00 ! Nobs =     1 ... Value =    117.79
DIHEdral CX31 CX26 CX27 CX23    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.99
DIHEdral CX31 CX26 CX27 HX41    2400.0 0     60.00 ! Nobs =     1 ... Value =     62.22
DIHEdral CX31 CX26 CX27 HX42    2400.0 0    -60.00 ! Nobs =     1 ... Value =    -62.20
DIHEdral CX25 CX26 CX31 CX30    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.06
DIHEdral CX25 CX26 CX31 HX39    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.96
DIHEdral CX27 CX26 CX31 CX30    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.94
DIHEdral CX27 CX26 CX31 HX39    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.04
DIHEdral CX25 CX28 CX29 CX30    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.01
DIHEdral CX25 CX28 CX29 HX38    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.95
DIHEdral HX37 CX28 CX29 CX30    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.97
DIHEdral HX37 CX28 CX29 HX38    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.07
DIHEdral CX28 CX29 CX30 CX31    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.03
DIHEdral CX28 CX29 CX30 NX32    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.98
DIHEdral HX38 CX29 CX30 CX31    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.93
DIHEdral HX38 CX29 CX30 NX32    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.02
DIHEdral CX29 CX30 CX31 CX26    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.06
DIHEdral CX29 CX30 CX31 HX39    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.95
DIHEdral NX32 CX30 CX31 CX26    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.99
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 DIHEdral NX32 CX30 CX31 HX39    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.09
 DIHEdral CX29 CX30 NX32 OX33    2400.0 0      0.00 ! Nobs =     1 ... Value =      0.03
 DIHEdral CX29 CX30 NX32 OX34    2400.0 0    180.00 ! Nobs =     1 ... Value =    179.92
 DIHEdral CX31 CX30 NX32 OX33    2400.0 0    180.00 ! Nobs =     1 ... Value =   -179.93
 DIHEdral CX31 CX30 NX32 OX34    2400.0 0      0.00 ! Nobs =     1 ... Value =     -0.04

!******************* end of mod by anda - HNF *************



{
!Dihedrals from param11.dna (included for terminal residues)

!DIHEdral     X     C2R   C3R   X       4.50 3     0.000
!DIHEdral     X     C4R   C3R   X       4.50 3     0.000
!DIHEdral     X     C2R   C1R   X       4.50 3     0.000
!DIHEdral     X     C5R   O5R   X       1.50 3     0.000
!DIHEdral     X     C3R   O3R   X       1.50 3     0.000
DIHEdral     X     C3R   OH    X       1.50 3     0.000
DIHEdral     X     C5R   OH    X       1.50 3     0.000
!DIHEdral     X     C2R   O2R   X       1.50 3     0.000
!DIHEdral     X     O5R   P     X       2.25 3     0.000
DIHEdral     X     OH    P     X       2.25 3     0.000
!DIHEdral     OH    C5R   C4R   O4R     4.50 3     0.000
!DIHEdral     OH    C5R   C4R   C3R     4.50 3     0.000 ! gamma
!DIHEdral     C3R   O3R   P     OH      2.25 3     0.000 ! added by infer
!DIHEdral     C3R   O3R   P     OH      2.25 2     0.000 ! ATB, 7-SEP-84
DIHEdral     C5R   O5R   P     OH      2.25 3     0.000 ! added by infer
!DIHEdral     C5R   O5R   P     OH      2.25 2     0.000 ! ATB, 7-SEP-84

!DIHEdral     X     C2D   C3D   X       4.50 3     0.000
!DIHEdral     X     C4D   C3D   X       4.50 3     0.000   !DNA
!DIHEdral     X     C2D   C1D   X       4.50 3     0.000
!DIHEdral     X     C5D   O5R   X       1.50 3     0.000
!DIHEdral     X     C3D   O3R   X       1.50 3     0.000
DIHEdral     X     C3D   OH    X       1.50 3     0.000
DIHEdral     X     C5D   OH    X       1.50 3     0.000
!DIHEdral     OH    C5D   C4D   O4D     4.50 3     0.000
!DIHEdral     OH    C5D   C4D   C3D     4.50 3     0.000
!DIHEdral     C3D   O3R   P     OH      2.25 3     0.000
!DIHEdral     C3D   O3R   P     OH      2.25 2     0.000
DIHEdral     C5D   O5R   P     OH      2.25 3     0.000
!DIHEdral     C5D   O5R   P     OH      2.25 2     0.000
}{
!Base hydrogen DIHEdrals taken from param11.dna
DIHEdral     X     C2G   N2G   X      18.0   2   180.000
DIHEdral     X     C6A   N6A   X      18.0   2   180.000
!DIHEdral     X     C6A   N4C   X      18.00 2   180.000
DIHEdral     X     C4C   N4C   X      18.00 2   180.000
}

!******************* mod by anda - HNF ********************

!!! IMPRoper PX1   OX2   OX3   OX4      750.0 0   -35.000 ! Nobs =     1 ... Value =   -36.223
 IMPRoper CX5   OX4   HX6   HX7      $kchimpr     0     32.868 ! mod by anda, gaussian value
 IMPRoper CX8   CX5   HX9   OX10      94.5 0   -36.228 ! mod by anda, gaussian value, k C5D  X     X
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          C2D
 IMPRoper CX11 OX10 CX14 OX18      94.5 0   -35.563 ! mod by anda, gaussian value
 IMPRoper CX12 CX8  HX13 CX14      94.5 0    41.651 ! mod by anda, gaussian value
 IMPRoper CX14 CX11 CX12 HX15      94.5 0    28.144 ! mod by anda, gaussian value
 IMPRoper CX19 OX18 CX20 CX24    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX20 CX19 CX21 HX36    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX21 CX20 CX22 HX35    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX22 CX21 CX23 CX25    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX23 CX22 CX24 CX27    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX24 CX19 CX23 HX40    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX25 CX22 CX26 CX28    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX26 CX25 CX27 CX31    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX27 CX23 CX26 HX41      94.5 0    28.808 ! mod by anda, gaussian value
 IMPRoper CX28 CX25 CX29 HX37    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX29 CX28 CX30 HX38    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX30 CX29 CX31 NX32    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX31 CX26 CX30 HX39    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper NX32 CX30 OX33 OX34    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX29 CX31 CX24 CX20    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX28 CX26 CX23 CX21    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper OX33 NX32 CX30 CX29    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper OX34 NX32 CX30 CX31    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper HX38 HX39 HX40 HX36    2400.0 0     0.000 ! mod by anda, gaussian value
 IMPRoper CX31 CX25 CX23 CX20    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX29 CX26 CX22 CX19    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX26 CX25 CX22 CX23    2400.0 0     0.000 ! mod by anda, planarity
 IMPRoper CX28 CX27 CX22 CX19    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX31 CX25 CX27 CX21    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper NX32 CX25 CX27 CX21    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX30 CX25 CX27 CX21    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX31 CX25 CX24 CX20    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper HX38 CX26 CX21 HX40    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper HX39 CX28 CX23 HX36    2400.0 0   180.000 ! mod by anda, planarity
 IMPRoper CX31 CX28 CX23 CX20    2400.0 0   180.000 ! mod by anda, planarity

!****************** end of mod by anda - HNF *************

!IMPRopers to keep the two purine rings parallel:
!guanine
IMPRoper     C8G   C4G   C5G   NNA   250.0   2   180.000
IMPRoper     C8G   C5G   C4G   C2G   250.0   2   180.000
IMPRoper     N3G   C4G   C5G   N7G   250.0   2   180.000
IMPRoper     C6G   C5G   C4G   N9G   250.0   2   180.000
!adenine
IMPRoper     C8A   C4A   C5A   N9A   250.0   2   180.000 ! WYE AND PATCHED RESIDUES
IMPRoper     C8A   C5A   C4A   C2A   250.0   2   180.000
IMPRoper     C8A   C4A   C5A   NC    250.0   2   180.000
IMPRoper     N3A   C4A   C5A   N7A   250.0   2   180.000
IMPRoper     C6A   C5A   C4A   N9A   250.0   2   180.000
!purine
IMPRoper     C8P   C4P   C5P   N9P   250.0   2   180.000 ! WYE AND PATCHED RESIDUES
IMPRoper     C8P   C5P   C4P   C2P   250.0   2   180.000
IMPRoper     C8P   C4P   C5P   NC    250.0   2   180.000
IMPRoper     N3P   C4P   C5P   N7P   250.0   2   180.000
IMPRoper     C6P   C5P   C4P   N9P   250.0   2   180.000
```

```
!other base specific non-exch hydrogen IMPRopers
IMPRoper    H    C4C  C6C  C5C  $kchimpr    0    0.000
IMPRoper    H    N1C  C5C  C6C  $kchimpr    0    0.000
IMPRoper    H    C4U  C6U  C5U  $kchimpr    0    0.000
IMPRoper    H    N1U  C5U  C6U  $kchimpr    0    0.000
IMPRoper    H    N1T  C5T  C6T  $kchimpr    0    0.000
IMPRoper    H    N7A  N9A  C8A  $kchimpr    0    0.000
IMPRoper    H    NC   N3A  C2A  $kchimpr    0    0.000
IMPRoper    H    N7P  N9P  C8P  $kchimpr    0    0.000
IMPRoper    H    NC   N3P  C2P  $kchimpr    0    0.000
IMPRoper    H    NC   C5P  C6P  $kchimpr    0    0.000
IMPRoper    H    N7G  N9G  C8G  $kchimpr    0    0.000

!Impropers for ribose chirality
IMPRoper    H    C2R  O4R  N9A    $kchimpr    0    -65.000!C1R
IMPRoper    H    C2R  O4R  N9P    $kchimpr    0    -65.000!C1R
IMPRoper    H    C2R  O4R  N9G    $kchimpr    0    -65.000!C1R
IMPRoper    H    C2R  O4R  N1C    $kchimpr    0    -65.000!C1R
IMPRoper    H    C2R  O4R  N1U    $kchimpr    0    -65.000!C1R
IMPRoper    H    C2R  O4R  N1T    $kchimpr    0    -65.000!C1R

IMPRoper    H    C3R  C1R  O2R    $kchimpr    0    65.000!C2R
IMPRoper    H    C4R  C2R  O3R    $kchimpr    0    60.300!C3R
IMPRoper    H    C4R  C2R  OH     $kchimpr    0    60.300!C3R; TERMINAL RES
IMPRoper    H    C5R  C3R  O4R    $kchimpr    0    70.300!C4R
IMPRoper    H    O5R  H    C4R    $kchimpr    0    72.000!C5R;
IMPRoper    H    OH   H    C4R    $kchimpr    0    72.000!C5R; TERMINAL RES
!Impropers for deoxyribose chirality
IMPRoper    H    C2D  O4D  N9A    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  N9P    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  N9G    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  N1C    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  N1T    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  N1U    $kchimpr    0    -65.280!C1D
IMPRoper    H    C2D  O4D  H      $kchimpr    0    -65.280!C1D !mod by anda (ABA)

IMPRoper    H    C3D  H    C1D    $kchimpr    0    -73.500!C2D
IMPRoper    H    C4D  C2D  O3R    $kchimpr    0    62.660!C3D
IMPRoper    H    C4D  C2D  OH     $kchimpr    0    62.660!C3D; TERMINAL RES
IMPRoper    H    C5D  C3D  O4D    $kchimpr    0    70.220!C4D
IMPRoper    H    O5R  H    C4D    $kchimpr    0    71.430!C5D;
IMPRoper    H    OH   H    C4D    $kchimpr    0    71.430!C5D; TERMINAL RES

{
!Phos. - periodical potentials from combined RNA/DNA statistics
!                                kq        x_eq       (sigma in parenthesis)
DIHEdral    O3R  P    O5R  C5R    1.41 3    24      ! alpha !P (20.3)
DIHEdral    P    O5R  C5R  C4R    3.45 0   178      ! beta  !P (13.0)
DIHEdral    O5R  C5R  C4R  C3R   12.24 3    18      ! gamma !S (6.9)
DIHEdral    O5R  C5R  C4R  O4R   24.28 3    14.1    !       !S (4.9)
DIHEdral    C4R  C3R  O3R  P      7.88 0  -153      ! eps   !P (8.6)
DIHEdral    C3R  O3R  P    O5R    1.75 3    33      ! zeta  !P (18.3)

DIHEdral    O3R  P    O5R  C5D    1.41 3     6.0 !DNA
DIHEdral    P    O5R  C5D  C4D    3.45 0   183.5
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DIHEdral    O5R  C5D  C4D  C3D   12.42 3   18.3
DIHEdral    O5R  C5D  C4D  O4D   24.28 3   14.1
DIHEdral    C4D  C3D  O3R  P      7.88 0  214.0
DIHEdral    C3D  O3R  P    O5R    1.75 3    0.3

!Phos. - discrete values from combined RNA/DNA statistics
!                                  kq       x_eq       (sigma in parenthesis)
!DIHEdral    O3R  P    O5R  C5R    6.07 0  285.3   ! (9.8)  alpha1 !P
!DIHEdral    O3R  P    O5R  C5R    3.98 0   81.1   ! (12.1) alpha2;alpha3=180.
!DIHEdral    P    O5R  C5R  C4R    3.44 0  183.5   ! (13.0) beta   !P
!DIHEdral    O5R  C5R  C4R  C3R   17.94 0   52.5   ! (5.7)  gamma1 !S
!DIHEdral    O5R  C5R  C4R  C3R   14.23 0  179.4   ! (6.4)  gamma2 !S
!DIHEdral    O5R  C5R  C4R  C3R    3.85 0  292.9   ! (12.3) gamma3 !S
!DIHEdral    C4R  C3R  O3R  P      7.88 0  214.0   ! (8.6)  eps    !P
!DIHEdral    C3R  O3R  P    O5R   25.30 0  289.2   ! (4.8)  zeta1  !P
!DIHEdral    C3R  O3R  P    O5R    2.85 0   80.7   ! (14.3) zeta2;zeta3=180.
}{
!Sugars
! c3'-endo conformation as the default for for RNA, c2'-endo for DNA,
!RNA statistics, C3'-endo
DIHEdral    C5R  C4R  C3R  O3R  30.12 0   81.1   ! delta ! c3'-endo S (4.4)
DIHEdral    O4R  C4R  C3R  O3R  33.10 0  201.8   !  4.2  ! c3'-endo S
DIHEdral    O4R  C1R  C2R  C3R  24.28 0  335.4   !  4.9  ! c3'-endo S
DIHEdral    C1R  C2R  C3R  C4R  74.36 0   35.9   !  2.8  ! c3'-endo S
DIHEdral    C2R  C3R  C4R  O4R  60.67 0  324.7   !  3.1  ! c3'-endo S
DIHEdral    C3R  C4R  O4R  C1R  22.42 0   20.5   !  5.1  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  C2R  15.67 0    2.8   !  6.1  ! c3'-endo S
DIHEdral    C5R  C4R  C3R  C2R  60.67 0  204.0   !  3.1  ! c3'-endo S
DIHEdral    O3R  C3R  C2R  O2R  28.79 0   44.3   !  4.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N1T  13.80 0  241.4   !  6.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N1C  13.80 0  241.4   !  6.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N1U  13.80 0  241.4   !  6.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N9G  13.80 0  241.4   !  6.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N9A  13.80 0  241.4   !  6.5  ! c3'-endo S
DIHEdral    C4R  O4R  C1R  N9P  13.80 0  241.4   !  6.5  ! c3'-endo S

!RNA c3'-endo sugar base joint torsions  (combined RNA/DNA statistics used)
DIHEdral    O4R  C1R  N1T  C2T  13.38 0  195.7   !  6.6  ! c3'-endo S
DIHEdral    O4R  C1R  N1C  C2C  13.38 0  195.7   !  6.6  ! c3'-endo S
DIHEdral    O4R  C1R  N1U  C2U  13.38 0  195.7   !  6.6  ! c3'-endo S
DIHEdral    O4R  C1R  N9A  C4A   2.97 0  193.3   ! 14.0  ! c3'-endo S
DIHEdral    O4R  C1R  N9P  C4P   2.97 0  193.3   ! 14.0  ! c3'-endo S
DIHEdral    O4R  C1R  N9G  C4G   2.97 0  193.3   ! 14.0  ! c3'-endo S

!DNA statistics (c2'-endo)
DIHEdral    C5D  C4D  C3D  O3R   36.44 0  145.2   ! delta ! c2'-endo S (4.0)
DIHEdral    O4D  C1D  C2D  C3D   24.28 0   32.8   !  4.9  ! c2'-endo S
DIHEdral    O4D  C4D  C3D  O3R   31.53 0  265.8   !  4.3  ! c2'-endo S
DIHEdral    C1D  C2D  C3D  C4D   44.99 0  326.9   !  3.6  ! c2'-endo S
DIHEdral    C2D  C3D  C4D  O4D   28.79 0   22.6   !  4.5  ! c2'-endo S
DIHEdral    C3D  C4D  O4D  C1D   15.67 0  357.7   !  6.1  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  C2D   14.69 0  340.7   !  6.3  ! c2'-endo S
DIHEdral    C5D  C4D  C3D  C2D   34.68 0  262.0   !  4.1  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  N1T   12.99 0  217.7   !  6.7  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  N1C   12.99 0  217.7   !  6.7  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  N1U   12.99 0  217.7   !  6.7  ! c2'-endo S
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DIHEdral    C4D  O4D  C1D  N9G   12.99 0  217.7   !  6.7  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  N9A   12.99 0  217.7   !  6.7  ! c2'-endo S
DIHEdral    C4D  O4D  C1D  N9P   12.99 0  217.7   !  6.7  ! c2'-endo S

!DNA c2'-endo sugar base joint torsions  (combined RNA/DNA statistics used)
DIHEdral    O4D  C1D  N1T  C2T    1.72 0  229.8   ! 18.4  ! c2'-endo S
DIHEdral    O4D  C1D  N1C  C2C    1.72 0  229.8   ! 18.4  ! c2'-endo S
DIHEdral    O4D  C1D  N1U  C2U    1.72 0  229.8   ! 18.4  ! c2'-endo S
DIHEdral    O4D  C1D  N9A  C4A    1.00 0  237.0   ! 24.3  ! c2'-endo S
DIHEdral    O4D  C1D  N9P  C4P    1.00 0  237.0   ! 24.3  ! c2'-endo S
DIHEdral    O4D  C1D  N9G  C4G    1.00 0  237.0   ! 24.3  ! c2'-endo S

!-------------------------------------------------------------------------
!In the case of c3'-endo conformation, the following DIHEdrals are provided
!to overwrite the c2'-endo DIHEdrals

!RNA statistics (c2'-endo)
!DIHEdral    C5R  C4R  C3R  O3R   24.28 0  147.3   ! delta ! c2'-endo S (4.9)
!DIHEdral    O4R  C1R  C2R  C3R   50.43 0   35.2   !  3.4  ! c2'-endo S
!DIHEdral    O4R  C4R  C3R  O3R   20.75 0  268.1   !  5.3  ! c2'-endo S
!DIHEdral    C1R  C2R  C3R  C4R   74.36 0  324.6   !  2.8  ! c2'-endo S
!DIHEdral    C2R  C3R  C4R  O4R   31.53 0   24.2   !  4.3  ! c2'-endo S
!DIHEdral    C3R  C4R  O4R  C1R   17.94 0  357.7   !  5.7  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  C2R   21.56 0  339.2   !  5.2  ! c2'-endo S
!DIHEdral    C5R  C4R  C3R  C2R   34.68 0  263.4   !  4.1  ! c2'-endo S
!DIHEdral    O3R  C3R  C2R  O2R   33.05 0  319.7   !  4.2  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  N1T   19.27 0  216.6   !  5.5  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  N1C   19.27 0  216.6   !  5.5  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  N1U   19.27 0  216.6   !  5.5  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  N9G   19.27 0  216.6   !  5.5  ! c2'-endo S
!DIHEdral    C4R  O4R  C1R  N9A   19.27 0  216.6   !  5.5  ! c2'-endo S

!RNA c2'-endo sugar base joint torsions  (combined RNA/DNA statistics used)
!DIHEdral    O4R  C1R  N1T  C2T    1.72 0  229.8   ! 18.4  ! c2'-endo S
!DIHEdral    O4R  C1R  N1C  C2C    1.72 0  229.8   ! 18.4  ! c2'-endo S
!DIHEdral    O4R  C1R  N1U  C2U    1.72 0  229.8   ! 18.4  ! c2'-endo S
!DIHEdral    O4R  C1R  N9A  C4A    1.00 0  237.0   ! 24.3  ! c2'-endo S
!DIHEdral    O4R  C1R  N9G  C4G    1.00 0  237.0   ! 24.3  ! c2'-endo S

!DNA statistics, c3'-endo (insuficient data, RNA values used)
!DIHEdral    C5D  C4D  C3D  O3R   30.12 0   81.1   ! delta ! c3'-endo S (4.4)
!DIHEdral    O4D  C4D  C3D  O3R   33.10 0  201.8   !  4.2  ! c3'-endo S
!DIHEdral    O4D  C1D  C2D  C3D   24.28 0  335.4   !  4.9  ! c3'-endo S
!DIHEdral    C1D  C2D  C3D  C4D   74.36 0   35.9   !  2.8  ! c3'-endo S
!DIHEdral    C2D  C3D  C4D  O4D   60.67 0  324.7   !  3.1  ! c3'-endo S
!DIHEdral    C3D  C4D  O4D  C1D   22.42 0   20.5   !  5.1  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  C2D   15.67 0    2.8   !  6.1  ! c3'-endo S
!DIHEdral    C5D  C4D  C3D  C2D   60.67 0  204.0   !  3.1  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  N1T   13.80 0  241.4   !  6.5  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  N1C   13.80 0  241.4   !  6.5  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  N1U   13.80 0  241.4   !  6.5  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  N9G   13.80 0  241.4   !  6.5  ! c3'-endo S
!DIHEdral    C4D  O4D  C1D  N9A   13.80 0  241.4   !  6.5  ! c3'-endo S

!DNA c3'-endo sugar base joint torsions  (combined RNA/DNA statistics used)
!DIHEdral    O4D  C1D  N1T  C2T   13.38 0  195.7   !  6.6  ! c3'-endo S
```

```
!DIHEdral     O4D   C1D   N1C   C2C   13.38 0   195.7    !   6.6   ! c3'-endo S
!DIHEdral     O4D   C1D   N1U   C2U   13.38 0   195.7    !   6.6   ! c3'-endo S
!DIHEdral     O4D   C1D   N9A   C4A    2.97 0   193.3    ! 14.0   ! c3'-endo S
!DIHEdral     O4D   C1D   N9G   C4G    2.97 0   193.3    ! 14.0   ! c3'-endo S
!-------------------------------------------------------------------------------
!-------------------------------------------------------------------------------
}
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!Impropers taken from param11.dna , 3*kq
IMPRoper      C5R   X     X     C2R    94.5   0    35.260
IMPRoper      C5R   X     X     C1R    94.5   0    35.260
IMPRoper      OH    X     X     C3R    94.5   0    35.260
IMPRoper      OH    X     X     C4R    94.5   0    35.260
IMPRoper      OH    X     X     C1R    94.5   0    35.260
IMPRoper      O3R   X     X     C3R    94.5   0    35.260
IMPRoper      O5R   X     X     C1R    94.5   0    35.260
IMPRoper      O2R   X     X     C2R    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N1T    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N1C    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N9G    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N9A    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N9P    94.5   0    35.260
IMPRoper      C5R   O4R   C3R   C4R    94.5   0    35.260
IMPRoper      N1T   C2R   O4R   C1R    94.5   0    35.260
IMPRoper      N1C   C2R   O4R   C1R    94.5   0    35.260
IMPRoper      N9A   C2R   O4R   C1R    94.5   0    35.260
IMPRoper      N9P   C2R   O4R   C1R    94.5   0    35.260
IMPRoper      N9G   C2R   O4R   C1R    94.5   0    35.260
IMPRoper      C4R   O5R   C1R   N1U    94.5   0    35.260
IMPRoper      N1U   C2R   O4R   C1R    94.5   0    35.260

IMPRoper      C5D   X     X     C2D    94.5   0    35.260   !DNA
IMPRoper      C5D   X     X     C1D    94.5   0    35.260
IMPRoper      OH    X     X     C3D    94.5   0    35.260
IMPRoper      OH    X     X     C4D    94.5   0    35.260
IMPRoper      OH    X     X     C1D    94.5   0    35.260
IMPRoper      O3R   X     X     C3D    94.5   0    35.260
IMPRoper      O5R   X     X     C1D    94.5   0    35.260
IMPRoper      C4D   O5R   C1D   N1T    94.5   0    35.260
IMPRoper      C4D   O5R   C1D   N1C    94.5   0    35.260
IMPRoper      C4D   O5R   C1D   N9G    94.5   0    35.260
IMPRoper      C4D   O5R   C1D   N9A    94.5   0    35.260
IMPRoper      C4D   O5R   C1D   N9P    94.5   0    35.260
IMPRoper      C5D   O4D   C3D   C4D    94.5   0    35.260
IMPRoper      N1T   C2D   O4D   C1D    94.5   0    35.260
IMPRoper      N1C   C2D   O4D   C1D    94.5   0    35.260
IMPRoper      N9A   C2D   O4D   C1D    94.5   0    35.260
IMPRoper      N9P   C2D   O4D   C1D    94.5   0    35.260
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IMPRoper     N9G   C2D   O4D   C1D    94.5   0    35.260
IMPRoper     C4D   O5R   C1D   N1U    94.5   0    35.260
IMPRoper     N1U   C2D   O4D   C1D    94.5   0    35.260

!the following impropers were taken from param11x.dna
!the higher kq was used to enforce the ring planarity
!cytosine
IMPRoper     C4C   X     X     ON   2400.0   0     0.000
IMPRoper     C4C   X     X     N1C   250.0   0     0.000
IMPRoper     C6C   X     X     NC    250.0   0     0.000
IMPRoper     C4C   X     X     N2   2400.0   0     0.000
IMPRoper     C2C   X     X     ON   2400.0   0     0.000
!infer
IMPRoper     C1R   C2C   C6C   N1C  2400.0   0     0.000
IMPRoper     C1D   C2C   C6C   N1C  2400.0   0     0.000
IMPRoper     N4C   NC    C5C   C4C  2400.0   0     0.000
IMPRoper     C2C   NC    C4C   C5C   250.0   0     0.000
IMPRoper     C5C   C6C   N1C   C2C   250.0   0     0.000
IMPRoper     H2    C4C   H2    N4C   250.0   0     0.000
IMPRoper     C5C   C4C   N4C   H2   2000.0   0     0.000

!uracil
IMPRoper     C4U   X     X     ON   2400.0   0     0.000
IMPRoper     C4U   X     X     N1U   250.0   0     0.000
IMPRoper     C6U   X     X     N3U   250.0   0     0.000
IMPRoper     C4U   X     X     N2   2400.0   0     0.000
IMPRoper     C2U   X     X     ON   2400.0   0     0.000
IMPRoper     C1R   C2U   C6U   N1U  2400.0   0     0.000
IMPRoper     C1D   C2U   C6U   N1U  2400.0   0     0.000
IMPRoper     ON    N3U   C5U   C4U   250.0   0     0.000
IMPRoper     C2U   N3U   C4U   C5U   250.0   0     0.000
IMPRoper     C5U   C6U   N1U   C2U   250.0   0     0.000
IMPRoper     H2    C4U   H2    ON    250.0   0     0.000
IMPRoper     HN    C2U   C4U   N3U   250.0   0     0.000
IMPRoper     H     C3D   NX29  C1D   500.0   0    -73.5 !added for pyr, jt

!thymidine
IMPRoper     C4T   X     X     ON   2400.0   0     0.000
IMPRoper     C4T   X     X     N1T   250.0   0     0.000
IMPRoper     C6T   X     X     N3T   250.0   0     0.000
IMPRoper     C4T   X     X     N2   2400.0   0     0.000
IMPRoper     C2T   X     X     ON   2400.0   0     0.000
IMPRoper     C1R   C2T   C6T   N1T  2400.0   0     0.000
IMPRoper     C1D   C2T   C6T   N1T  2400.0   0     0.000
IMPRoper     ON    N3T   C5T   C4T   250.0   0     0.000
IMPRoper     C2T   N3T   C4T   C5T   250.0   0     0.000
IMPRoper     C5T   C6T   N1T   C2T   250.0   0     0.000
IMPRoper     H2    C4T   H2    ON    250.0   0     0.000
IMPRoper     CC3E  C4T   C6T   C5T  2400.0   0     0.000

!infer
IMPRoper     HN    C2T   C4T   N3T   250.0   0     0.000

! The ring-spanning impropers have been left out.
!adenine
IMPRoper     N2A   N3A   NC    C2A   250.0   0     0.000
```

```
IMPRoper     H2   C2A  H2   N2A   250.0  0    0.000
IMPRoper     C4A  C5A  N7A  C8A   250.0  0    0.000
IMPRoper     C5A  C4A  N9A  C8A   250.0  0    0.000
IMPRoper     C4A  X    X    NC    250.0  0    0.000
IMPRoper     C2A  X    X    N9A   250.0  0    0.000
IMPRoper     C2A  X    X    C5A   250.0  0    0.000
IMPRoper     C6A  C5A  C4A  N3A   250.0  0    0.000
IMPRoper     C5A  X    X    N9A   250.0  0    0.000
IMPRoper     C6A  X    X    N6A  2400.0  0    0.000
IMPRoper     H2   X    X    N6A   250.0  0    0.000

!infer
IMPRoper     C1R  C4A  C8A  N9A  2400.0  0    0.000
IMPRoper     C1D  C4A  C8A  N9A  2400.0  0    0.000
IMPRoper     N9A  C4A  C5A  N7A   250.0  0    0.000
IMPRoper     N7A  C8A  N9A  C4A   250.0  0    0.000
IMPRoper     N3A  C2A  NC   C6A   250.0  0    0.000
IMPRoper     C5A  C6A  N6A  H2   2000.0  0    0.000

! The ring-spanning impropers have been left out.
!purine
IMPRoper     N2P  N3P  NC   C2P   250.0  0    0.000
IMPRoper     C4P  C5P  N7P  C8P   250.0  0    0.000
IMPRoper     C5P  C4P  N9P  C8P   250.0  0    0.000
IMPRoper     C4P  X    X    NC    250.0  0    0.000
IMPRoper     C2P  X    X    N9P   250.0  0    0.000
IMPRoper     C2P  X    X    C5P   250.0  0    0.000
IMPRoper     C6P  C5P  C4P  N3P   250.0  0    0.000
IMPRoper     C5P  X    X    N9P   250.0  0    0.000

!infer
IMPRoper     C1R  C4P  C8P  N9P  2400.0  0    0.000
IMPRoper     C1D  C4P  C8P  N9P  2400.0  0    0.000
IMPRoper     N9P  C4P  C5P  N7P   250.0  0    0.000
IMPRoper     N7P  C8P  N9P  C4P   250.0  0    0.000
IMPRoper     N3P  C2P  NC   C6P   250.0  0    0.000

! The ring-spanning impropers have been left out.
!guanine
IMPRoper     C4G  C5G  N7G  C8G   250.0  0    0.000
IMPRoper     C5G  C4G  N9G  C8G   250.0  0    0.000
IMPRoper     C4G  X    X    NNA   250.0  0    0.000
IMPRoper     C2G  X    X    N9G   250.0  0    0.000
IMPRoper     C2G  X    X    C5G   250.0  0    0.000
IMPRoper     C6G  C5G  C4G  N3G   250.0  0    0.000
IMPRoper     C5G  X    X    N9G   250.0  0    0.000
IMPRoper     C6G  X    X    O6G  2400.0  0    0.000
IMPRoper     C2G  X    X    N2G  2400.0  0    0.000

!infer
IMPRoper     C1R  C4G  C8G  N9G  2400.0  0    0.000
IMPRoper     C1D  C4G  C8G  N9G  2400.0  0    0.000
IMPRoper     N9G  C4G  C5G  N7G   250.0  0    0.000
IMPRoper     N7G  C8G  N9G  C4G   250.0  0    0.000
IMPRoper     N3G  C2G  NNA  C6G   250.0  0    0.000
IMPRoper     H2   H2   C2G  N2G   250.0  0    0.000
```

```
IMPRoper     HN   C2G  C6G  NNA 2000.0  0     0.000
IMPRoper     N3G  C2G  N2G  H2  2000.0  0     0.000

!                    Lennard-Jones parameters
!                                          ------1-4-------
!                   epsilon    sigma        epsilon    sigma
!                  (Kcal/mol)    (A)       (Kcal/mol) (A)
! Taken from Rossky Karplus and Rahman BIOPOLY (1979)
! 0.05 ADDED TO RADII TO IMPRoperOVE ON NUCL.ACID STACKING/LN
!
!                    eps      sigma        eps(1:4) sigma(1:4)

!******************* mod by anda - HNF ********************

 NONBonded PX1  0.5849  3.3854    0.5849  3.3854 ! mod by anda, std value
 NONBonded OX2  0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded OX3  0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded OX4  0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded CX5  0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded HX6  0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX7  0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded CX8  0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded HX9  0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded OX10 0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded CX11 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX12 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded HX13 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded CX14 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded HX15 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX16 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded OX17 0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded OX18 0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded CX19 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX20 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX21 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX22 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX23 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX24 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX25 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX26 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX27 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX28 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX29 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX30 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded CX31 0.0900  3.2970    0.0900  3.2970 ! mod by anda, std value
 NONBonded NX32 0.1600  2.8591    0.1600  2.8591 ! mod by anda, std value
 NONBonded OX33 0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded OX34 0.2304  2.7290    0.2304  2.7290 ! mod by anda, std value
 NONBonded HX35 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX36 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX37 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX38 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX39 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX40 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX41 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
 NONBonded HX42 0.0045  2.6160    0.0045  2.6160 ! mod by anda, std value
```

```
 NONBonded HX43 0.0045   2.6160     0.0045   2.6160 ! mod by anda, std value

!******************* end of mod by anda - HNF *************

NONBonded   C5R      0.0900    3.2970       0.0900    3.2970
NONBonded   C1R      0.0900    3.2970       0.0900    3.2970
NONBonded   C2R      0.0900    3.2970       0.0900    3.2970
NONBonded   C3R      0.0900    3.2970       0.0900    3.2970
NONBonded   C4R      0.0900    3.2970       0.0900    3.2970

NONBonded   C5D      0.0900    3.2970       0.0900    3.2970   !DNA
NONBonded   C1D      0.0900    3.2970       0.0900    3.2970
NONBonded   C2D      0.0900    3.2970       0.0900    3.2970
NONBonded   C3D      0.0900    3.2970       0.0900    3.2970
NONBonded   C4D      0.0900    3.2970       0.0900    3.2970

NONBonded   HN       0.0045    2.6160       0.0045    2.6160
NONBonded   H2       0.0045    1.6040       0.0045    1.6040
NONBonded   H        0.0045    2.6160       0.0045    2.6160
!
! give it the same as th Hn from RKR
NONBonded   HO       0.0045    1.6040       0.0045    1.6040

!
! THIS STILL IS AN EXTENDED ATOM
NONBonded   O3R       0.2304    2.7290       0.2304    2.7290
NONBonded   O4R       0.2304    2.7290       0.2304    2.7290
NONBonded   O4D       0.2304    2.7290       0.2304    2.7290
NONBonded   O5R       0.2304    2.7290       0.2304    2.7290
NONBonded   O1P       0.2304    2.7290       0.2304    2.7290
NONBonded   O2P       0.2304    2.7290       0.2304    2.7290
NONBonded   P         0.5849    3.3854       0.5849    3.3854

!bases
NONBonded   C2       0.0900    3.2970       0.0900    3.2970
NONBonded   C3       0.0900    3.2970       0.0900    3.2970
NONBonded   CB       0.0900    3.2970       0.0900    3.2970
NONBonded   CE       0.0900    3.2970       0.0900    3.2970
NONBonded   CH       0.0900    3.2970       0.0900    3.2970

NONBonded   N2       0.1600    2.8591       0.1600    2.8591
NONBonded   N3U      0.1600    2.8591       0.1600    2.8591
NONBonded   N3T      0.1600    2.8591       0.1600    2.8591
NONBonded   NNA      0.1600    2.8591       0.1600    2.8591
NONBonded   NB       0.1600    2.8591       0.1600    2.8591
NONBonded   NC       0.1600    2.8591       0.1600    2.8591

NONBonded   NH2E     0.1600    3.0291       0.1600    3.0291
NONBonded   NS       0.1600    2.8591       0.1600    2.8591
NONBonded   N1T      0.1600    2.8591       0.1600    2.8591
NONBonded   N1C      0.1600    2.8591       0.1600    2.8591
NONBonded   N9A      0.1600    2.8591       0.1600    2.8591
NONBonded   N9P      0.1600    2.8591       0.1600    2.8591
NONBonded   N9G      0.1600    2.8591       0.1600    2.8591
NONBonded   N1U      0.1600    2.8591       0.1600    2.8591
NONBonded   ON       0.2304    2.7290       0.2304    2.7290
```

```
NONBonded  O2R      0.2304   2.7290      0.2304   2.7290
NONBonded  OH       0.2304   2.5508      0.2304   2.5508
NONBonded  SD       0.3515   2.6727      0.3515   2.6727  ! G U E S S
NONBonded  O2       0.2304   2.7290      0.2304   2.7290

! NEW
NONBonded  C6C      0.0900   3.2970      0.0900   3.2970
NONBonded  C5C      0.0900   3.2970      0.0900   3.2970
NONBonded  C4C      0.0900   3.2970      0.0900   3.2970
NONBonded  C2C      0.0900   3.2970      0.0900   3.2970
NONBonded  C6U      0.0900   3.2970      0.0900   3.2970
NONBonded  C5U      0.0900   3.2970      0.0900   3.2970
NONBonded  C4U      0.0900   3.2970      0.0900   3.2970
NONBonded  C2U      0.0900   3.2970      0.0900   3.2970
NONBonded  C8A      0.0900   3.2970      0.0900   3.2970
NONBonded  C6A      0.0900   3.2970      0.0900   3.2970
NONBonded  C5A      0.0900   3.2970      0.0900   3.2970
NONBonded  C4A      0.0900   3.2970      0.0900   3.2970
NONBonded  C2A      0.0900   3.2970      0.0900   3.2970
NONBonded  C8P      0.0900   3.2970      0.0900   3.2970
NONBonded  C6P      0.0900   3.2970      0.0900   3.2970
NONBonded  C5P      0.0900   3.2970      0.0900   3.2970
NONBonded  C4P      0.0900   3.2970      0.0900   3.2970
NONBonded  C2P      0.0900   3.2970      0.0900   3.2970
NONBonded  C8G      0.0900   3.2970      0.0900   3.2970
NONBonded  C6G      0.0900   3.2970      0.0900   3.2970
NONBonded  C5G      0.0900   3.2970      0.0900   3.2970
NONBonded  C4G      0.0900   3.2970      0.0900   3.2970
NONBonded  C2G      0.0900   3.2970      0.0900   3.2970
NONBonded  C6T      0.0900   3.2970      0.0900   3.2970
NONBonded  C5T      0.0900   3.2970      0.0900   3.2970
NONBonded  C4T      0.0900   3.2970      0.0900   3.2970
NONBonded  C2T      0.0900   3.2970      0.0900   3.2970
NONBonded  N4C      0.1600   2.8591      0.1600   2.8591
NONBonded  O4U      0.2304   2.7290      0.2304   2.7290
NONBonded  N7G      0.1600   2.8591      0.1600   2.8591
NONBonded  N3G      0.1600   2.8591      0.1600   2.8591
NONBonded  N2G      0.1600   2.8591      0.1600   2.8591
NONBonded  N3A      0.1600   2.8591      0.1600   2.8591
NONBonded  N7A      0.1600   2.8591      0.1600   2.8591
NONBonded  N6A      0.1600   2.8591      0.1600   2.8591
NONBonded  O6G      0.2304   2.7290      0.2304   2.7290
NONBonded  CC3E     0.0900   3.2970      0.0900   3.2970
NONBonded  N2A      0.1600   2.8591      0.1600   2.8591
NONBonded  N2P      0.1600   2.8591      0.1600   2.8591
NONBonded  N3P      0.1600   2.8591      0.1600   2.8591
NONBonded  N7P      0.1600   2.8591      0.1600   2.8591

! special solute-solute hydrogen bonding potential parameters
!AEXP 4
!REXP 6

!HAEX 4

!AAEX 2
! "all" possible combinations of HB-pairs in nucleic acids:
```

Script code

```
!  WELL DEPTHS DEEPENED BY 0.5 KCAL TO IMPROVE BASEPAIR ENERGIES /LN
!  AND DISTANCES INCREASED BY 0.05
!                        Emin        Rmin
!                    (Kcal/mol)    (A)
!hbond  N*   O*        -14.0         2.95
!hbond  N*   N*        -14.5         3.05
!hbond  O*   O*        -15.75        2.80
!hbond  O*   N*        -15.50        2.90

! the following NBFIXes are for DNA-DNA hydrogen bonding

! terms
!                                                  ------1-4------
!                      A             B             A             B
!             [Kcal/(mol A^12)] [Kcal/(mol A^6)]
!
nbfix   HO    ON       0.05          0.1           0.05          0.1
nbfix   HO    O3R      0.05          0.1           0.05          0.1
nbfix   HO    O5R      0.05          0.1           0.05          0.1
nbfix   HO    OH       0.05          0.1           0.05          0.1
nbfix   HO    O2R      0.05          0.1           0.05          0.1
nbfix   HO    NC       0.05          0.1           0.05          0.1


nbfix   H     ON       0.05          0.1           0.05          0.1
nbfix   H     O2       0.05          0.1           0.05          0.1
nbfix   H     O5R      0.05          0.1           0.05          0.1
nbfix   H     O4R      0.05          0.1           0.05          0.1
nbfix   H     O4D      0.05          0.1           0.05          0.1
nbfix   H     O3R      0.05          0.1           0.05          0.1
nbfix   H     O2R      0.05          0.1           0.05          0.1
nbfix   H     OH       0.05          0.1           0.05          0.1
nbfix   H     N7A      0.05          0.1           0.05          0.1
nbfix   H     N7P      0.05          0.1           0.05          0.1
nbfix   H     N7G      0.05          0.1           0.05          0.1
nbfix   H     N3A      0.05          0.1           0.05          0.1
nbfix   H     N3P      0.05          0.1           0.05          0.1
nbfix   H     N3G      0.05          0.1           0.05          0.1


nbfix   HN    ON       0.05          0.1           0.05          0.1
nbfix   HN    O2R      0.05          0.1           0.05          0.1
nbfix   HN    OH       0.05          0.1           0.05          0.1
nbfix   HN    NC       0.05          0.1           0.05          0.1

nbfix   H2    ON       0.05          0.1           0.05          0.1
nbfix   H2    O2R      0.05          0.1           0.05          0.1
nbfix   H2    OH       0.05          0.1           0.05          0.1

nbfix   H2    NC       0.05          0.1           0.05          0.1

!mod by anda-----------------------------------------------------------------------------------------------

!2-AP
BOND          C2P     N2G          1110.000     1.341 !0.010   B
ANGLe          NC     C2P     N2G          449.866   116.20   !0.90 B
```

```
ANGLe      N3P    C2P    N2G        743.656  119.900 !0.70 B
ANGLe      C2P    N2G    H2         105.000  120.000  !from param11.dna, 3*keq
DIHEdral   X    C2P  N2G  X     18.0  2  180.000
IMPRoper   H2   H2   C2P  N2G  250.0  0    0.000
IMPRoper   N3P  C2P  N2G  H2  2000.0  0    0.000
IMPRoper   C2P  X    X    N2G 2400.0  0    0.000

!end of mod by
     anda-------------------------------------------------------------------------------------------

set echo=on message=on end



  !RNA TOPOLOGY FILE 'FRAMEWORK' FROM TOPALLHDG.DNA AND ATOM NAMES
    !  FROM DNA-RNA.PARAM
    !INCLUDES ALL NONEXCHANGEABLE HYDROGENS AND TERMS FOR BOND, ANGLE, AND
    !IMPROPERS. NONEXCHANGEABLE HYDROGEN CHARGES WERE ASSIGNED 0.035.
    !CARBON CHARGES WERE REDUCED 0.035 FOR EACH ATTACHED HYDROGEN.
    !CREATED 2/24/96-- JASON P. RIFE AND PETER B. MOORE
    ! DNA-RNA-ALLATOM.TOP

    set echo=false end


    ! checkversion 1.0


    AUTOGENERATE ANGLES=TRUE END
    {*============================*}

!******************** mod by anda - HNF ********************

 MASS PX1    30.97400 ! assuming P -> 30.97400 + 1.008 * 0 (Hs)
 MASS OX2    15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS OX3    15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS OX4    15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS CX5    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS HX6     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX7     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS CX8    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS HX9     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS OX10   15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS CX11   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX12   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS HX13    1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS CX14   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS HX15    1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX16    1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS OX17   15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS OX18   15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS CX19   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX20   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX21   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX22   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX23   12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
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 MASS CX24    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX25    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX26    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX27    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX28    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX29    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX30    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS CX31    12.01100 ! assuming C -> 12.01100 + 1.008 * 0 (Hs)
 MASS NX32    14.00700 ! assuming N -> 14.00700 + 1.008 * 0 (Hs)
 MASS OX33    15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS OX34    15.99900 ! assuming O -> 15.99900 + 1.008 * 0 (Hs)
 MASS HX35     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX36     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX37     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX38     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX39     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX40     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX41     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX42     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)
 MASS HX43     1.00800 ! assuming H -> 1.00800 + 1.008 * 0 (Hs)

!******************** end of mod by anda - HNF *************

    {* DNA/RNA default masses *}

    MASS    P       30.97400! phosphorus
    MASS    O1P     15.99940! O in phosphate
    MASS    O2P     15.99940! O in phosphate
    MASS    O5R     15.99940! ester -P-O-C-
    MASS    C5R     12.011! corresp. to CH2E
    MASS    C4R     12.011! corresp. to CH1E
    MASS    C3R     12.011! corresp. to CH1E
    MASS    C2R     12.011! corresp. to CH1E
    MASS    C1R     12.011! corresp. to CH1E
    MASS    O4R     15.99940! ester -P-O-C-
    MASS    O3R     15.99940! ester -P-O-C-
    MASS    O2R     15.99940! ester -P-O-C-
    MASS    OH      15.99940! corresp. to OH1

    !DEOXY SUGAR
    MASS    C5D     14.02700! corresp. to CH2E
    MASS    C4D     13.01900! corresp. to CH1E
    MASS    C3D     13.01900! corresp. to CH1E
    MASS    C2D     13.01900! corresp. to CH1E
    MASS    C1D     13.01900! corresp. to CH1E
    MASS    O4D     15.99940! ester -P-O-C-
    MASS    O5D     15.99940!
    MASS    O3D     15.99940!

    ! Insert Bases
    ! Generic

    MASS    N2      14.00670! nitrogen in -NH2
    MASS    NNA     14.00670! corresp. to NH1
    MASS    ON      15.99940! corresp. to O
    MASS    NC      14.00670! corresp. to NR
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```
MASS    NS     14.00670! nitrogen in ring >N-

! Insert 4 Bases
!  GUA
MASS    N9G     14.00670! nitrogen in ring >N-
MASS    C2G     12.011! (prev CE)
MASS    N3G     14.00670! (prev NC)
MASS    C4G     12.01100! (prev CB)
MASS    C5G     12.01100! (prev CB)
MASS    C6G     12.01100! (prev CN)
MASS    N7G     14.00670! (prev NB)
MASS    C8G     12.011! (prev CE)
MASS    O6G     15.99940! (prev CE)
MASS    N2G     14.00670! nitrogen in -NH2
!  ADE
MASS    N9A     14.00670! nitrogen in ring >N-
MASS    C2A     12.011! (prev CE)
MASS    N3A     14.00670! (prev NC)
MASS    C4A     12.01100! (prev CB)
MASS    C5A     12.01100! (prev CB)
MASS    C6A     12.01100! (prev CA)
MASS    N7A     14.00670! (prev NB)
MASS    C8A     12.011! (prev CE)
MASS    N6A     14.00670! nitrogen in -NH2

! PUR
MASS    N9P     14.00670! nitrogen in ring >N-
MASS    C2P     12.011! (prev CE)
MASS    N3P     14.00670! (prev NC)
MASS    C4P     12.01100! (prev CB)
MASS    C5P     12.01100! (prev CB)
MASS    C6P     12.01100! (prev CA)
MASS    N7P     14.00670! (prev NB)
MASS    C8P     12.011! (prev CE)

!  CYT
MASS    N1C     14.00670! nitrogen in ring >N-
MASS    C2C     12.01100! (prev CN)
MASS    C4C     12.01100! (prev CA)
MASS    C5C     12.011! (prev CF)
MASS    C6C     12.011! (prev CF)
MASS    N4C     14.00670! nitrogen in -NH2

!  THY
MASS    N1T     14.00670! nitrogen in ring >N-
MASS    N3T     14.00670! nitrogen in ring >N-
MASS    C2T     12.01100! (prev CN)
MASS    C4T     12.01100! (prev CN)
MASS    C5T     12.011! (prev CS)
MASS    C6T     12.011! (prev CF)
MASS    CC3E    12.01100! (prev CF)

! END

MASS    H       1.00800! non-exchangeable Hydrogens
MASS    HN      1.00800! corresp. to H
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```
    MASS    H2      1.00800! hydrogen in -NH2
    MASS    HO      1.00800! hydroxy hydrogen

    !  URI
    MASS    N1U     14.00670! nitrogen in ring >N-
    MASS    C2U     12.01100! (prev CN)
    MASS    C4U     12.01100! (prev CA)
    MASS    C5U     12.011! (prev CF)
    MASS    C6U     12.011! (prev CF)
    MASS    N3U     14.00670!


    !-------------------------------------------------------------------------
    !-------------------------------------------------------------------------

!******************** mod by anda - HNF ****************************

RESIdue HNF

 { Note: electrostatics should normally not be used in }
 { crystallographic refinement since it can produce }
 { artefacts. For this reason, all charges are set to }
 { zero by default. Edit them if necessary }

GROUp
 ATOM  P     TYPE PX1   CHARGE=1.20    END
 ATOM  O1P   TYPE OX2   CHARGE=-0.40   END
 ATOM  O2P   TYPE OX3   CHARGE=-0.40   END
 ATOM  O5'   TYPE OX4   CHARGE=-0.36   END
GROUp
 ATOM  C5'   TYPE CX5   CHARGE=-0.070  END
 ATOM H5'    TYPE HX6   CHARGE=0.035   END
 ATOM H5''   TYPE HX7   CHARGE=0.035   END
GROUp
 ATOM  C4'   TYPE CX8   CHARGE=0.100   END !mod. to match DFT calc
 ATOM  H4'   TYPE HX9   CHARGE=0.105   END !mod. to match DFT calc
 ATOM  O4'   TYPE OX10  CHARGE=-0.263  END !mod. to match DFT calc, inc. by +0.13 for neutrality
 ATOM  C1'   TYPE CX11  CHARGE=0.302   END !mod. to match DFT calc
 ATOM  H1''  TYPE HX43  CHARGE=0.076   END !mod. to match DFT calc
 ATOM  O2    TYPE OX18  CHARge=-0.244  END !reduced charge by 0.076 (residual charge of HNF)
GROUp
 ATOM  C2'   TYPE CX14  CHARGE=-0.070   END
 ATOM H2'    TYPE HX15  CHARGE=0.035   END
 ATOM H2''   TYPE HX16  CHARGE=0.035   END
GROUp
 ATOM  C3'   TYPE CX12  CHARGE=-0.035  END
 ATOM  H3'   TYPE HX13  CHARGE=0.035   END
GROUp
 ATOM  O3'   TYPE OX17  CHARGE=-0.36   END
!HNF-base
GROUp
 ATOM  C2    TYPE CX19  CHARge   0.449  END
 ATOM  C3    TYPE CX20  CHARge  -0.372  END
 ATOM  C4    TYPE CX21  CHARge  -0.089  END
 ATOM  C10   TYPE CX22  CHARge  -0.119  END
 ATOM  C11   TYPE CX23  CHARge   0.225  END
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ATOM  C1   TYPE CX24  CHARge  -0.447  END
ATOM  C13  TYPE CX25  CHARge   0.078  END
ATOM  C12  TYPE CX26  CHARge   0.134  END
ATOM  C9   TYPE CX27  CHARge  -0.191  END
ATOM  C5   TYPE CX28  CHARge  -0.154  END
ATOM  C6   TYPE CX29  CHARge  -0.230  END
ATOM  C7   TYPE CX30  CHARge   0.078  END
ATOM  C8   TYPE CX31  CHARge  -0.317  END
ATOM  N7   TYPE NX32  CHARge   0.751  END
ATOM  O71  TYPE OX33  CHARge  -0.453  END
ATOM  O72  TYPE OX34  CHARge  -0.456  END
ATOM  H4   TYPE HX35  CHARge   0.147  END
ATOM  H3   TYPE HX36  CHARge   0.156  END
ATOM  H5   TYPE HX37  CHARge   0.141  END
ATOM  H6   TYPE HX38  CHARge   0.181  END
ATOM  H8   TYPE HX39  CHARge   0.193  END
ATOM  H1   TYPE HX40  CHARge   0.102  END
ATOM  H91  TYPE HX41  CHARge   0.105  END
ATOM  H92  TYPE HX42  CHARge   0.164  END
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BOND  P     O1P      BOND  P     O2P      BOND  P     O5'      BOND  O5'  C5'
BOND  C5'  H5'       BOND  C5'  H5''     BOND  C5'  C4'      BOND  C4'  H4'
BOND  C4'  O4'       BOND  C4'  C3'      BOND  O4'  C1'      BOND  C1'  C2'
BOND  C1'  O2        BOND  C1'  H1''      BOND  C3'  H3'       BOND  C3'  C2'
BOND  C3'  O3'       BOND  C2'  H2'      BOND  C2'  H2''     BOND  O2   C2
BOND  C2   C3        BOND  C2   C1       BOND  C3   C4       BOND  C3   H3
BOND  C4   C10       BOND  C4   H4       BOND  C10  C11      BOND  C10  C13
BOND  C11  C1        BOND  C11  C9       BOND  C1   H1       BOND  C13  C12
BOND  C13  C5        BOND  C12  C9       BOND  C12  C8       BOND  C9   H91
BOND  C9   H92       BOND  C5   C6       BOND  C5   H5       BOND  C6   C7
BOND  C6   H6        BOND  C7   C8       BOND  C7   N7       BOND  C8   H8
BOND  N7   O71       BOND  N7   O72
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```
{dihedrals taken out in accord with other bases}
{
 { Note: edit these DIHEdrals if necessary }
  DIHEdral  O2P  P    O5'  C5' ! flat ? (180 degrees = trans)   172.75
! DIHEdral  P    O5'  C5'  H5'  ! flexible dihedral ???   -63.48
! DIHEdral  P    O5'  C5'  H5'' ! flexible dihedral ???    55.20
  DIHEdral  P    O5'  C5'  C4' ! flat ? (180 degrees = trans)   176.08
  DIHEdral  O5'  C5'  C4'  H4' ! flat ? (180 degrees = trans)   172.35
! DIHEdral  O5'  C5'  C4'  O4' ! flexible dihedral ???   -69.24
! DIHEdral  O5'  C5'  C4'  C3' ! flexible dihedral ???    52.07
! DIHEdral H5'   C5'  C4'  H4' ! flexible dihedral ???    51.91
  DIHEdral H5'   C5'  C4'  O4' ! flat ? (180 degrees = trans)   170.31
! DIHEdral H5'   C5'  C4'  C3' ! flexible dihedral ???   -68.38
! DIHEdral H5'' C5'  C4'  H4' ! flexible dihedral ???   -66.18
! DIHEdral H5'' C5'  C4'  O4' ! flexible dihedral ???    52.23
  DIHEdral H5'' C5'  C4'  C3' ! flat ? (180 degrees = trans)   173.54
! DIHEdral  C5'  C4'  C3'  C2' ! flexible dihedral ??? -129.37
! DIHEdral  C5'  C4'  C3'  O3' ! flexible dihedral ???   112.32
! DIHEdral  H4'  C4'  C3'  C2' ! flexible dihedral ???   110.44
  DIHEdral  H4'  C4'  C3'  O3' ! flat ? (0 degrees = cis)    -7.87
! DIHEdral  O4'  C4'  C3'  H3' ! flexible dihedral ???   111.59
  DIHEdral  O4'  C4'  C3'  C2' ! flat ? (0 degrees = cis)    -5.92
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! DIHEdral  O4'   C4'   C3'   O3' ! flexible dihedral ???   -124.23
! DIHEdral  O2    C1'   C2' H2'   ! flexible dihedral ???     80.69
  DIHEdral  O4'   C1'   O2    C2   ! flat ? (180 degrees = trans)    177.32
! DIHEdral  C2'   C1'   O2    C2   ! flexible dihedral ???    -67.60
! DIHEdral  H1''  C1'   O2    C2   ! flexible dihedral ???     56.28
! DIHEdral  C4'   C3'   C2' H2'' ! flexible dihedral ???    -88.75
! DIHEdral  H3'   C3'   C2'   C1' ! flexible dihedral ???    -90.38
! DIHEdral  O3'   C3'   C2' H2'   ! flexible dihedral ???    -93.06}
  DIHEdral  O2    C2    C3    C4   ! flat ? (180 degrees = trans)    180.39
  DIHEdral  O2    C2    C3    H3   ! flat ? (0 degrees = cis)      -0.12
  DIHEdral  C1    C2    C3    C4   ! flat ? (0 degrees = cis)      -0.46
  DIHEdral  C1    C2    C3    H3   ! flat ? (180 degrees = trans)    179.03
  DIHEdral  O2    C2    C1    C11  ! flat ? (180 degrees = trans)    179.62
  DIHEdral  O2    C2    C1    H1   ! flat ? (0 degrees = cis)      -0.53
  DIHEdral  C3    C2    C1    C11  ! flat ? (0 degrees = cis)       0.42
  DIHEdral  C3    C2    C1    H1   ! flat ? (180 degrees = trans)    180.27
  DIHEdral  C2    C3    C4    C10  ! flat ? (0 degrees = cis)       0.26
  DIHEdral  C2    C3    C4    H4   ! flat ? (180 degrees = trans)    180.16
  DIHEdral  H3    C3    C4    C10  ! flat ? (180 degrees = trans)    180.76
  DIHEdral  H3    C3    C4    H4   ! flat ? (0 degrees = cis)       0.67
  DIHEdral  C3    C4    C10   C11  ! flat ? (0 degrees = cis)      -0.02
  DIHEdral  C3    C4    C10   C13  ! flat ? (180 degrees = trans)    179.94
  DIHEdral  H4    C4    C10   C11  ! flat ? (180 degrees = trans)    180.08
  DIHEdral  H4    C4    C10   C13  ! flat ? (0 degrees = cis)       0.03
  DIHEdral  C4    C10   C11   C1   ! flat ? (0 degrees = cis)      -0.02
  DIHEdral  C4    C10   C11   C9   ! flat ? (180 degrees = trans)    179.89
  DIHEdral  C13   C10   C11   C1   ! flat ? (180 degrees = trans)    180.02
  DIHEdral  C13   C10   C11   C9   ! flat ? (0 degrees = cis)      -0.07
  DIHEdral  C4    C10   C13   C12  ! flat ? (180 degrees = trans)    180.10
  DIHEdral  C4    C10   C13   C5   ! flat ? (0 degrees = cis)       0.02
  DIHEdral  C11   C10   C13   C12  ! flat ? (0 degrees = cis)       0.06
  DIHEdral  C11   C10   C13   C5   ! flat ? (180 degrees = trans)    179.98
  DIHEdral  C10   C11   C1    C2   ! flat ? (0 degrees = cis)      -0.18
  DIHEdral  C10   C11   C1    H1   ! flat ? (180 degrees = trans)    179.97
  DIHEdral  C9    C11   C1    C2   ! flat ? (180 degrees = trans)    179.93
  DIHEdral  C9    C11   C1    H1   ! flat ? (0 degrees = cis)       0.08
  DIHEdral  C10   C11   C9    C12  ! flat ? (0 degrees = cis)       0.05
! DIHEdral  C10   C11   C9    H91  ! flexible dihedral ???    117.87
! DIHEdral  C10   C11   C9    H92  ! flexible dihedral ???   -117.81
  DIHEdral  C1    C11   C9    C12  ! flat ? (180 degrees = trans)    179.95
! DIHEdral  C1    C11   C9    H91  ! flexible dihedral ???    -62.23
! DIHEdral  C1    C11   C9    H92  ! flexible dihedral ???     62.09
  DIHEdral  C10   C13   C12   C9   ! flat ? (0 degrees = cis)      -0.03
  DIHEdral  C10   C13   C12   C8   ! flat ? (180 degrees = trans)    179.97
  DIHEdral  C5    C13   C12   C9   ! flat ? (180 degrees = trans)    180.04
  DIHEdral  C5    C13   C12   C8   ! flat ? (0 degrees = cis)       0.04
  DIHEdral  C10   C13   C5    C6   ! flat ? (180 degrees = trans)    180.08
  DIHEdral  C10   C13   C5    H5   ! flat ? (0 degrees = cis)       0.06
  DIHEdral  C12   C13   C5    C6   ! flat ? (0 degrees = cis)      -0.01
  DIHEdral  C12   C13   C5    H5   ! flat ? (180 degrees = trans)    179.97
  DIHEdral  C13   C12   C9    C11  ! flat ? (0 degrees = cis)      -0.01
! DIHEdral  C13   C12   C9    H91  ! flexible dihedral ???   -117.79
! DIHEdral  C13   C12   C9    H92  ! flexible dihedral ???    117.79
  DIHEdral  C8    C12   C9    C11  ! flat ? (180 degrees = trans)    179.99
! DIHEdral  C8    C12   C9    H91  ! flexible dihedral ???     62.22
! DIHEdral  C8    C12   C9    H92  ! flexible dihedral ???    -62.20
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 DIHEdral  C13  C12  C8   C7  ! flat ? (0 degrees = cis)     -0.06
 DIHEdral  C13  C12  C8   H8  ! flat ? (180 degrees = trans)   180.04
 DIHEdral  C9   C12  C8   C7  ! flat ? (180 degrees = trans)   179.94
 DIHEdral  C9   C12  C8   H8  ! flat ? (0 degrees = cis)      0.04
 DIHEdral  C13  C5   C6   C7  ! flat ? (0 degrees = cis)      0.01
 DIHEdral  C13  C5   C6   H6  ! flat ? (180 degrees = trans)   180.05
 DIHEdral  H5   C5   C6   C7  ! flat ? (180 degrees = trans)   180.03
 DIHEdral  H5   C5   C6   H6  ! flat ? (0 degrees = cis)      0.07
 DIHEdral  C5   C6   C7   C8  ! flat ? (0 degrees = cis)     -0.03
 DIHEdral  C5   C6   C7   N7  ! flat ? (180 degrees = trans)   180.02
 DIHEdral  H6   C6   C7   C8  ! flat ? (180 degrees = trans)   179.93
 DIHEdral  H6   C6   C7   N7  ! flat ? (0 degrees = cis)     -0.02
 DIHEdral  C6   C7   C8   C12 ! flat ? (0 degrees = cis)      0.06
 DIHEdral  C6   C7   C8   H8  ! flat ? (180 degrees = trans)   179.95
 DIHEdral  N7   C7   C8   C12 ! flat ? (180 degrees = trans)   180.01
 DIHEdral  N7   C7   C8   H8  ! flat ? (0 degrees = cis)     -0.09
 DIHEdral  C6   C7   N7   O71 ! flat ? (0 degrees = cis)      0.03
 DIHEdral  C6   C7   N7   O72 ! flat ? (180 degrees = trans)   179.92
 DIHEdral  C8   C7   N7   O71 ! flat ? (180 degrees = trans)   180.07
 DIHEdral  C8   C7   N7   O72 ! flat ? (0 degrees = cis)     -0.04


 { Note: edit these IMPRopers if necessary }
!!! IMPRoper  P    O1P  O2P  O5' ! chirality or flatness improper   -36.22 ! taken out in
     accordance with other bases
 IMPRoper  C5'  O5' H5'  H5'' ! chirality or flatness improper    -34.21
 IMPRoper  C4'  C5'  H4'  O4' ! chirality or flatness improper    -37.05
 IMPRoper  C1'  O4'  C2'  O2  ! chirality or flatness improper    -33.04
 IMPRoper  C3'  C4'  H3'  C2' ! chirality or flatness improper     41.55
 IMPRoper  C2'  C1'  C3' H2'  ! chirality or flatness improper     28.65
 IMPRoper  C2   O2   C3   C1  ! chirality or flatness improper      0.51
 IMPRoper  C3   C2   C4   H3  ! chirality or flatness improper     -0.27
 IMPRoper  C4   C3   C10  H4  ! chirality or flatness improper     -0.05
 IMPRoper  C10  C4   C11  C13 ! chirality or flatness improper     -0.03
 IMPRoper  C11  C10  C1   C9  ! chirality or flatness improper     -0.06
 IMPRoper  C1   C2   C11  H1  ! chirality or flatness improper     -0.08
 IMPRoper  C13  C10  C12  C5  ! chirality or flatness improper     -0.04
 IMPRoper  C12  C13  C9   C8  ! chirality or flatness improper      0.00
 IMPRoper  C9   C11  C12  H91 ! chirality or flatness improper    -29.65
 IMPRoper  C5   C13  C6   H5  ! chirality or flatness improper     -0.01
 IMPRoper  C6   C5   C7   H6  ! chirality or flatness improper      0.02
 IMPRoper  C7   C6   C8   N7  ! chirality or flatness improper      0.03
 IMPRoper  C8   C12  C7   H8  ! chirality or flatness improper      0.05
 IMPRoper  N7   C7   O71  O72 ! chirality or flatness improper     -0.06
 IMPRoper  C6   C8   C1   C3  ! chirality or flatness improper     -0.01
 IMPRoper  C5   C12  C11  C4  ! chirality or flatness improper      0.02
 IMPRoper  O71  N7   C7   C6  ! chirality or flatness improper      0.03
 IMPRoper  O72  N7   C7   C8  ! chirality or flatness improper      0.05
 IMPRoper  H6   H8   H1   H3  ! chirality or flatness improper     -0.06
 IMPRoper  C8   C13  C11  C3  ! mod by anda, planarity
 IMPRoper  C6   C12  C10  C2  ! mod by anda, planarity
 IMPRoper  C12  C13  C10  C11 ! mod by anda, planarity
 IMPRoper  C5   C9   C10  C2  ! mod by anda, planarity
 IMPRoper  C8   C13  C9   C4  ! mod by anda, planarity
 IMPRoper  N7   C13  C9   C4  ! mod by anda, planarity
 IMPRoper  C7   C13  C9   C4  ! mod by anda, planarity
```

```
 IMPRoper  C8   C13  C1   C3  ! mod by anda, planarity
 IMPRoper  H6   C12  C4   H1  ! mod by anda, planarity
 IMPRoper  H8   C5   C11  H3  ! mod by anda, planarity
 IMPRoper  C8   C5   C11  C3  ! mod by anda, planarity

!   { Note: edit any DONOrs and ACCEptors if necessary }
!  ! DONOr H?  O1P ! only true if -OHx (x>0)
!   ACCEptor  O1P  P
!  ! DONOr H?  O2P ! only true if -OHx (x>0)
!   ACCEptor  O2P  P
!   ACCEptor  O5'  P
!   ACCEptor  O4'  C4'
!  ! DONOr H?  O3' ! only true if -OHx (x>0)
!   ACCEptor  O3'  C3'
!   ACCEptor  O2   C1'
!  ! DONOr H?  O71 ! only true if -OHx (x>0)
!   ACCEptor  O71  N7
!  ! DONOr H?  O72 ! only true if -OHx (x>0)
!   ACCEptor  O72  N7

END { RESIdue HNF }

!******************** end of mod by anda - HNF ********************


    RESIdue GUA
      GROUp
      ATOM P     TYPE=P     CHARGE=1.20    END
      ATOM O1P   TYPE=O1P   CHARGE=-0.40   END
      ATOM O2P   TYPE=O2P   CHARGE=-0.40   END
      ATOM O5'   TYPE=O5R   CHARGE=-0.36   END
     GROUp
      ATOM C5'   TYPE=C5R   CHARGE=-0.070  END
      ATOM H5'   TYPE=H     CHARGE=0.035   END !JPR
      ATOM H5''  TYPE=H     CHARGE=0.035   END !JPR
     GROUp
      ATOM C4'   TYPE=C4R   CHARGE=0.065  END
      ATOM H4'   TYPE=H     CHARGE=0.035   END !JPR
      ATOM O4'   TYPE=O4R   CHARGE=-0.30   END
      ATOM C1'   TYPE=C1R   CHARGE=0.165   END !JPR
      ATOM H1'   TYPE=H     CHARGE=0.035   END !JPR

    ! Insert Base
     GROUp
      ATOM N9    TYPE=N9G   CHARGE=-0.19   END
      ATOM C4    TYPE=C4G   CHARGE=0.19    EXCLusion=( N1 )   END
     GROUp
      ATOM N3    TYPE=N3G   CHARGE=-0.35   EXCLusion=( C6 )   END
      ATOM C2    TYPE=C2G   CHARGE=0.35    EXCLusion=( C5 )   END
     GROUp
      ATOM N2    TYPE=N2G   CHARGE=-0.42   END
      ATOM H21   TYPE=H2    CHARGE=0.21    END
      ATOM H22   TYPE=H2    CHARGE=0.21    END
     GROUp
      ATOM N1    TYPE=NNA   CHARGE=-0.26   END
      ATOM H1    TYPE=HN    CHARGE=0.26    END
```

```
 GROUp
  ATOM C6    TYPE=C6G    CHARGE=0.30     END
  ATOM O6    TYPE=O6G    CHARGE=-0.30    END
 GROUp
  ATOM C5    TYPE=C5G    CHARGE=0.02     END
  ATOM N7    TYPE=N7G    CHARGE=-0.25    END
  ATOM C8    TYPE=C8G    CHARGE=0.145    END
  ATOM H8    TYPE=H      CHARGE=0.035    END

!

 GROUP
  ATOM C2'   TYPE=C2R    CHARGE=0.115    END
  ATOM H2'   TYPE=H      CHARGE=0.035    END
  ATOM O2'   TYPE=O2R    CHARGE=-0.40    END
  ATOM HO2'  TYPE=HO     CHARGE=0.25     END
 GROUP
  ATOM C3'   TYPE=C3R     CHARGE=-0.035  END
  ATOM H3'   TYPE=H       CHARGE=0.035   END
 GROUP
  ATOM O3'   TYPE=O3R    CHARGE=-0.36    END

 BOND P     O1P          BOND P     O2P          BOND P     O5'

 BOND O5'   C5'          BOND C5'   C4'          BOND C4'   O4'
 BOND C4'   C3'          BOND O4'   C1'          BOND C1'   N9
 BOND C1'   C2'          BOND N9    C4           BOND N9    C8
 BOND C4    N3           BOND C4    C5           BOND N3    C2
 BOND C2    N2           BOND C2    N1           BOND N2    H21

 BOND N2    H22          BOND N1    H1           BOND N1    C6
 BOND C6    O6           BOND C6    C5           BOND C5    N7
 BOND N7    C8           BOND C2'   C3'          BOND C3'   O3'
 BOND C2'   O2'          BOND C8    H8

 BOND O2'   HO2'
 BOND C5'   H5'          BOND C5'   H5''         BOND C4'   H4'
 BOND C3'   H3'          BOND C2'   H2'          BOND C1'   H1'
{
 DIHEdral   P     O5'   C5'   C4'        DIHEdral   O5'   C5'   C4'   O4'
 DIHEdral   O5'   C5'   C4'   C3'
}{
 DIHEdral   C3'   C4'   O4'   C1'
 DIHEdral   C4'   O4'   C1'   C2'        DIHEdral   O4'   C1'   C2'   C3'

 DIHEdral   C1'   C2'   C3'   C4'        DIHEdral   O4'   C4'   C3'   O3'
 DIHEdral   C5'   C4'   C3'   C2'        DIHEdral   O3'   C3'   C2'   O2'
 DIHEdral   O4'   C1'   N9    C4
 DIHEdral   C3'   C2'   O2'   H2'
}

 !
 IMPRoper N3    C2    N2    H21         IMPRoper C1'   C4    C8    N9
 IMPRoper N9    C4    C5    N7          IMPRoper C4    C5    N7    C8
 IMPRoper C5    N7    C8    N9          IMPRoper N7    C8    N9    C4
 IMPRoper C8    N9    C4    C5          IMPRoper N2    N3    N1    C2
```

```
 IMPRoper H1   C2   C6   N1          IMPRoper O6   N1   C5   C6
 IMPRoper C4   N3   C2   N1          IMPRoper N3   C2   N1   C6
 IMPRoper C2   N1   C6   C5          IMPRoper N1   C6   C5   C4

 IMPRoper C6   C5   C4   N3          IMPRoper C5   C4   N3   C2
 IMPRoper H22  H21  C2   N2
 IMPRoper H8   N7   N9   C8

!IMPRoper to keep the two purine rings parallel:
 IMPRoper C8   C4   C5   N1          IMPRoper C8   C5   C4   C2
 IMPRoper N3   C4   C5   N7          IMPRoper C6   C5   C4   N9

!RIBOSE IMPROPERS
IMPRoper      H1'  C2'  O4'  N9  !C1'
IMPRoper      H2'  C3'  C1'  O2' !C2'
IMPRoper      H3'  C4'  C2'  O3' !C3'
IMPRoper      H4'  C5'  C3'  O4' !C4'
IMPRoper      H5'  O5'  H5'' C4' !C5'



 END {GUA}

! ---------------------------------------------------------------------------

RESIdue ADE
GROUp
   ATOM P     TYPE=P     CHARGE=1.20    END
   ATOM O1P   TYPE=O1P   CHARGE=-0.40   END
   ATOM O2P   TYPE=O2P   CHARGE=-0.40   END
   ATOM O5'   TYPE=O5R   CHARGE=-0.36   END
 GROUp
   ATOM C5'   TYPE=C5R   CHARGE=-0.070  END
   ATOM H5'   TYPE=H     CHARGE=0.035   END
   ATOM H5''  TYPE=H     CHARGE=0.035   END
 GROUp
   ATOM C4'   TYPE=C4R   CHARGE=0.065   END
   ATOM H4'   TYPE=H     CHARGE=0.035   END
   ATOM O4'   TYPE=O4R   CHARGE=-0.30   END
   ATOM C1'   TYPE=C1R   CHARGE=0.165   END
   ATOM H1'   TYPE=H     CHARGE=0.035   END

! Insert Base
 GROUp
   ATOM N9    TYPE=N9A   CHARGE=-0.19   END
   ATOM C4    TYPE=C4A   CHARGE=0.19    EXCLusion=( N1 )  END
 GROUp
   ATOM N3    TYPE=N3A   CHARGE=-0.26   EXCLusion=( C6 )  END
   ATOM C2    TYPE=C2A   CHARGE=0.225   EXCLusion=( C5 )  END
   ATOM H2    TYPE=H     CHARGE=0.035   END
 GROUp
   ATOM N1    TYPE=NC    CHARGE=-0.28   END
   ATOM C6    TYPE=C6A   CHARGE=0.28    END
 GROUp
   ATOM N6    TYPE=N6A   CHARGE=-0.42   END
```

```
  ATOM H61    TYPE=H2     CHARGE=0.21     END
  ATOM H62    TYPE=H2     CHARGE=0.21     END
 GROUp
  ATOM C5     TYPE=C5A    CHARGE=0.02     END
  ATOM N7     TYPE=N7A    CHARGE=-0.25    END
  ATOM C8     TYPE=C8A    CHARGE=0.195    END
  ATOM H8     TYPE=H      CHARGE=0.035    END
! END

 GROUP
  ATOM C2'   TYPE=C2R    CHARGE=0.115    END
  ATOM H2'   TYPE=H      CHARGE=0.035    END
  ATOM O2'   TYPE=O2R    CHARGE=-0.40    END
  ATOM HO2'  TYPE=HO     CHARGE=0.25     END
 GROUP
  ATOM C3'   TYPE=C3R    CHARGE=-0.035   END
  ATOM H3'   TYPE=H      CHARGE=0.035    END
 GROUP
  ATOM O3'   TYPE=O3R    CHARGE=-0.36    END

 BOND P     O1P              BOND P     O2P              BOND P     O5'
 BOND O5'   C5'              BOND C5'   C4'              BOND C4'   O4'
 BOND C4'   C3'              BOND O4'   C1'              BOND C1'   N9
 BOND C1'   C2'              BOND N9    C4               BOND N9    C8
 BOND C4    N3               BOND C4    C5               BOND N3    C2
 BOND C2    N1               BOND N1    C6               BOND C6    N6

 BOND N6    H61              BOND N6    H62              BOND C6    C5

 BOND C5    N7               BOND N7    C8               BOND C2'   C3'
 BOND C2'   O2'              BOND C3'   O3'
 BOND C8    H8               BOND C2    H2
 BOND O2'   HO2'
 BOND C5'   H5'         BOND C5'   H5''         BOND C4'   H4'
 BOND C3'   H3'         BOND C2'   H2'         BOND C1'   H1'
{
 DIHEdral P    O5'   C5'   C4'            DIHEdral O5'   C5'   C4'   O4'
 DIHEdral O5'  C5'   C4'   C3'
}{
 DIHEdral C3'  C4'   O4'   C1'
 DIHEdral C4'  O4'   C1'   C2'            DIHEdral O4'   C1'   C2'   C3'

 DIHEdral C1'  C2'   C3'   C4'            DIHEdral O4'   C4'   C3'   O3'
 DIHEdral C5'  C4'   C3'   C2'            DIHEdral O2'   C2'   C3'   O3'
 DIHEdral O4'  C1'   N9    C4
 DIHEdral C3'  C2'   O2'   H2'
 }

 !
 IMPRoper C5    C6    N6    H61            IMPRoper C1'   C4    C8    N9
 IMPRoper N9    C4    C5    N7             IMPRoper C4    C5    N7    C8
 IMPRoper C5    N7    C8    N9             IMPRoper N7    C8    N9    C4
 IMPRoper C8    N9    C4    C5             IMPRoper N6    N1    C5    C6
 IMPRoper H62   C6    H61   N6             IMPRoper C4    N3    C2    N1
 IMPRoper N3    C2    N1    C6             IMPRoper C2    N1    C6    C5
 IMPRoper N1    C6    C5    C4             IMPRoper C6    C5    C4    N3
```

```
 IMPRoper C5   C4   N3   C2
 IMPRoper H2   N1   N3   C2              IMPRoper H8   N7   N9   C8
 ! IMPRoper to keep the two purine rings parallel:
 IMPRoper C8   C4   C5   N1              IMPRoper C8   C5   C4   C2
 IMPRoper N3   C4   C5   N7              IMPRoper C6   C5   C4   N9


!RIBOSE IMPROPERS
IMPRoper      H1'  C2'  O4'  N9  !C1'
IMPRoper      H2'  C3'  C1'  O2' !C2'
IMPRoper      H3'  C4'  C2'  O3' !C3'
IMPRoper      H4'  C5'  C3'  O4' !C4'
IMPRoper      H5'  O5'  H5'' C4' !C5'



 END {ADE}

! ------------------------------------------------------------------------

RESIdue PUR
GROUp
   ATOM P     TYPE=P     CHARGE=1.20    END
   ATOM O1P   TYPE=O1P   CHARGE=-0.40   END
   ATOM O2P   TYPE=O2P   CHARGE=-0.40   END
   ATOM O5'   TYPE=O5R   CHARGE=-0.36   END
 GROUp
   ATOM C5'   TYPE=C5R   CHARGE=-0.070  END
   ATOM H5'   TYPE=H     CHARGE=0.035   END
   ATOM H5''  TYPE=H     CHARGE=0.035   END
 GROUp
   ATOM C4'   TYPE=C4R   CHARGE=0.065   END
   ATOM H4'   TYPE=H     CHARGE=0.035   END
   ATOM O4'   TYPE=O4R   CHARGE=-0.30   END
   ATOM C1'   TYPE=C1R   CHARGE=0.165   END
   ATOM H1'   TYPE=H     CHARGE=0.035   END

! Insert Base
 GROUp
   ATOM N9    TYPE=N9P   CHARGE=-0.19   END
   ATOM C4    TYPE=C4P   CHARGE=0.19    EXCLusion=( N1 )  END
 GROUp
   ATOM N3    TYPE=N3P   CHARGE=-0.26   EXCLusion=( C6 )  END
   ATOM C2    TYPE=C2P   CHARGE=0.225   EXCLusion=( C5 )  END
   ATOM H2    TYPE=H     CHARGE=0.035   END
 GROUp
   ATOM N1    TYPE=NC    CHARGE=-0.28   END
   ATOM C6    TYPE=C6P   CHARGE=0.28    END
   ATOM H6    TYPE=H     CHARge= 0.035  END
 GROUp
   ATOM C5    TYPE=C5P   CHARGE=0.02    END
   ATOM N7    TYPE=N7P   CHARGE=-0.25   END
   ATOM C8    TYPE=C8P   CHARGE=0.195   END
   ATOM H8    TYPE=H     CHARGE=0.035   END
! END
```

```
 GROUP
  ATOM C2'   TYPE=C2R    CHARGE=0.115    END
  ATOM H2'   TYPE=H      CHARGE=0.035    END
  ATOM O2'   TYPE=O2R    CHARGE=-0.40    END
  ATOM HO2'  TYPE=HO     CHARGE=0.25     END
 GROUP
  ATOM C3'   TYPE=C3R    CHARGE=-0.035   END
  ATOM H3'   TYPE=H      CHARGE=0.035    END
 GROUP
  ATOM O3'   TYPE=O3R    CHARGE=-0.36    END

 BOND P     O1P              BOND P     O2P              BOND P     O5'
 BOND O5'   C5'              BOND C5'   C4'              BOND C4'   O4'
 BOND C4'   C3'              BOND O4'   C1'              BOND C1'   N9
 BOND C1'   C2'              BOND N9    C4               BOND N9    C8
 BOND C4    N3               BOND C4    C5               BOND N3    C2
 BOND C2    N1               BOND N1    C6               BOND C6    H6

 BOND C6    C5

 BOND C5    N7               BOND N7    C8               BOND C2'   C3'
 BOND C2'   O2'              BOND C3'   O3'
 BOND C8    H8               BOND C2    H2
 BOND O2'   HO2'
 BOND C5'   H5'         BOND C5'   H5''         BOND C4'   H4'
 BOND C3'   H3'         BOND C2'   H2'         BOND C1'   H1'
{
 DIHEdral P    O5'  C5'  C4'            DIHEdral O5'  C5'  C4'  O4'
 DIHEdral O5'  C5'  C4'  C3'
}{
 DIHEdral C3'  C4'  O4'  C1'
 DIHEdral C4'  O4'  C1'  C2'            DIHEdral O4'  C1'  C2'  C3'

 DIHEdral C1'  C2'  C3'  C4'            DIHEdral O4'  C4'  C3'  O3'
 DIHEdral C5'  C4'  C3'  C2'            DIHEdral O2'  C2'  C3'  O3'
 DIHEdral O4'  C1'  N9   C4
 DIHEdral C3'  C2'  O2'  H2'
 }

 !
 IMPRoper H6   N1   C5   C6             IMPRoper C1'  C4   C8   N9
 IMPRoper N9   C4   C5   N7             IMPRoper C4   C5   N7   C8
 IMPRoper C5   N7   C8   N9             IMPRoper N7   C8   N9   C4
 IMPRoper C8   N9   C4   C5             IMPRoper N6   N1   C5   C6
 IMPRoper C4   N3   C2   N1
 IMPRoper N3   C2   N1   C6             IMPRoper C2   N1   C6   C5
 IMPRoper N1   C6   C5   C4             IMPRoper C6   C5   C4   N3
 IMPRoper C5   C4   N3   C2
 IMPRoper H2   N1   N3   C2             IMPRoper H8   N7   N9   C8
 ! IMPRoper to keep the two purine rings parallel:
 IMPRoper C8   C4   C5   N1             IMPRoper C8   C5   C4   C2
 IMPRoper N3   C4   C5   N7             IMPRoper C6   C5   C4   N9


!RIBOSE IMPROPERS
IMPRoper      C2'  C3'  C1'  O2'
```

```
IMPRoper      H1'   C2'   O4'   N9   !C1'
IMPRoper      H2'   C3'   C1'   O2'  !C2'
IMPRoper      H3'   C4'   C2'   O3'  !C3'
IMPRoper      H4'   C5'   C3'   O4'  !C4'
IMPRoper      H5'   O5'   H5''  C4'  !C5'



 END {PUR}

! ---------------------------------------------------------------------------

RESIdue ABA
  GROUp
  ATOM P     TYPE=P     CHARGE=1.20     END
  ATOM O1P   TYPE=O1P   CHARGE=-0.40    END
  ATOM O2P   TYPE=O2P   CHARGE=-0.40    END
  ATOM O5'   TYPE=O5R   CHARGE=-0.36    END
 GROUp
  ATOM C5'   TYPE=C5R   CHARGE=-0.070   END
  ATOM H5'   TYPE=H     CHARGE=0.035    END
  ATOM H5''  TYPE=H     CHARGE=0.035    END
 GROUp
  ATOM C4'   TYPE=C4R   CHARGE=0.065    END
  ATOM H4'   TYPE=H     CHARGE=0.035    END
  ATOM O4'   TYPE=O4R   CHARGE=-0.30    END
  ATOM C1'   TYPE=C1R   CHARGE=0.165    END
  ATOM H1'   TYPE=H     CHARGE=0.018    END
  ATOM H1''  TYPE=H     CHARGE=0.017    END

GROUP
  ATOM C2'   TYPE=C2R   CHARGE=0.115    END
  ATOM H2'   TYPE=H     CHARGE=0.035    END
  ATOM O2'   TYPE=O2R   CHARGE=-0.40    END
  ATOM HO2'  TYPE=HO    CHARGE=0.25     END
 GROUP
  ATOM C3'   TYPE=C3R   CHARGE=-0.035   END
  ATOM H3'   TYPE=H     CHARGE=0.035    END
 GROUP
  ATOM O3'   TYPE=O3R   CHARGE=-0.36    END

 BOND P    O1P          BOND P    O2P          BOND P    O5'
 BOND O5'  C5'          BOND C5'  C4'          BOND C4'  O4'
 BOND C4'  C3'          BOND O4'  C1'
 BOND C1'  C2'          BOND C2'  C3'
 BOND C3'  O3'          BOND C2'  O2'
 BOND O2'  HO2'         BOND C1'  H1''
 BOND C5'  H5'          BOND C5'  H5''         BOND C4'  H4'
 BOND C3'  H3'          BOND C2'  H2'          BOND C1'  H1'

{
 DIHEdral P    O5'  C5'  C4'          DIHEdral O5'  C5'  C4'  O4'
 DIHEdral O5'  C5'  C4'  C3'
}{
 DIHEdral C3'  C4'  O4'  C1'
 DIHEdral C4'  O4'  C1'  C2'          DIHEdral O4'  C1'  C2'  C3'
```

```
 DIHEdral C1'  C2'  C3'  C4'            DIHEdral O4'  C4'  C3'  O3'
 DIHEdral C5'  C4'  C3'  C2'            DIHEdral O2'  C2'  C3'  O3'
 DIHEdral O4'  C1'  H1'' C2
 DIHEdral C3'  C2'  O2'  H2'

 ! New dihedrals
 DIHEdral  C5'  C4'  C3'  O3'           DIHEdral  C4'  O4'  C1'  H1''
}

    !RIBOSE IMPROPERS
!IMPRoper     H1'  C2'  O4'  H1''  !C1' !mod by anda
IMPRoper      H2'  C3'  C1'  O2' !C2'
IMPRoper      H3'  C4'  C2'  O3' !C3'
IMPRoper      H4'  C5'  C3'  O4' !C4'
IMPRoper      H5'  O5'  H5'' C4' !C5'



 END {ABA}


! ------------------------------------------------------------------------

RESIdue CYT
  GROUp
  ATOM P     TYPE=P     CHARGE=1.20    END
  ATOM O1P   TYPE=O1P   CHARGE=-0.40   END
  ATOM O2P   TYPE=O2P   CHARGE=-0.40   END
  ATOM O5'   TYPE=O5R   CHARGE=-0.36   END
 GROUp
  ATOM C5'   TYPE=C5R   CHARGE=-0.070  END
  ATOM H5'   TYPE=H     CHARGE=0.035   END
  ATOM H5''  TYPE=H     CHARGE=0.035   END
 GROUp
  ATOM C4'   TYPE=C4R   CHARGE=0.065   END
  ATOM H4'   TYPE=H     CHARGE=0.035   END
  ATOM O4'   TYPE=O4R   CHARGE=-0.30   END
  ATOM C1'   TYPE=C1R   CHARGE=0.165   END
  ATOM H1'   TYPE=H     CHARGE=0.035   END

! Insert Base

 GROUp
  ATOM N1    TYPE=N1C   CHARGE=-0.19  EXCLUSION=( C4 )  END
  ATOM C6    TYPE=C6C   CHARGE=0.155  EXCLUSION=( N3 )  END
  ATOM H6    TYPE=H     CHARGE=0.035  END
 GROUp
  ATOM C2    TYPE=C2C   CHARGE=0.30    EXCLUSION=( C5 )  END
  ATOM O2    TYPE=ON    CHARGE=-0.30   END
 GROUp
  ATOM N3    TYPE=NC    CHARGE=-0.28   END
  ATOM C4    TYPE=C4C   CHARGE=0.28    END
 GROUp
  ATOM N4    TYPE=N4C   CHARGE=-0.42   END
  ATOM H41   TYPE=H2    CHARGE=0.21    END
```

```
  ATOM H42    TYPE=H2     CHARGE=0.21     END
 GROUp
  ATOM C5     TYPE=C5C    CHARGE=-0.035   END !CHRG
  ATOM H5     TYPE=H      CHARGE=0.035    END
 GROUp

! END


GROUP
  ATOM C2'   TYPE=C2R   CHARGE=0.115   END
  ATOM H2'   TYPE=H     CHARGE=0.035   END
  ATOM O2'   TYPE=O2R   CHARGE=-0.40   END
  ATOM HO2'  TYPE=HO    CHARGE=0.25    END
 GROUP
  ATOM C3'   TYPE=C3R   CHARGE=-0.035  END
  ATOM H3'   TYPE=H     CHARGE=0.035   END
 GROUP
  ATOM O3'   TYPE=O3R   CHARGE=-0.36   END

 BOND P     O1P              BOND P     O2P              BOND P     O5'
 BOND O5'   C5'              BOND C5'   C4'              BOND C4'   O4'
 BOND C4'   C3'              BOND O4'   C1'              BOND C1'   N1
 BOND C1'   C2'              BOND N1    C2               BOND N1    C6
                             BOND C2    N3               BOND N3    C4
 BOND C4    N4               BOND N4    H41              BOND N4    H42
 BOND C2    O2
 BOND C4    C5               BOND C5    C6               BOND C2'   C3'
 BOND C3'   O3'              BOND C2'   O2'
 BOND C6    H6               BOND C5    H5
 BOND O2'   HO2'
 BOND C5'   H5'          BOND C5'   H5''          BOND C4'   H4'
 BOND C3'   H3'          BOND C2'   H2'          BOND C1'   H1'

{
 DIHEdral P     O5'   C5'   C4'            DIHEdral O5'   C5'   C4'   O4'
 DIHEdral O5'   C5'   C4'   C3'
}{
 DIHEdral C3'   C4'   O4'   C1'
 DIHEdral C4'   O4'   C1'   C2'            DIHEdral O4'   C1'   C2'   C3'

 DIHEdral C1'   C2'   C3'   C4'            DIHEdral O4'   C4'   C3'   O3'
 DIHEdral C5'   C4'   C3'   C2'            DIHEdral O2'   C2'   C3'   O3'
 DIHEdral O4'   C1'   N1    C2
 DIHEdral C3'   C2'   O2'   H2'

 ! New dihedrals
 DIHEdral   C5'   C4'   C3'   O3'          DIHEdral   C4'   O4'   C1'   N1
}

 IMPRoper C5    C4    N4    H41             IMPRoper C1'   C2    C6    N1
 IMPRoper O2    N1    N3    C2              IMPRoper N4    N3    C5    C4
 IMPRoper N1    C2    N3    C4              IMPRoper C2    N3    C4    C5
 IMPRoper N3    C4    C5    C6              IMPRoper C4    C5    C6    N1
 IMPRoper C5    C6    N1    C2              IMPRoper C6    N1    C2    N3
 IMPRoper H42   C4    H41   N4
```

```
 IMPRoper H5   C4   C6    C5                    IMPRoper   H6    N1    C5    C6

!RIBOSE IMPROPERS
IMPRoper       H1'   C2'   O4'   N1   !C1'
IMPRoper       H2'   C3'   C1'   O2'  !C2'
IMPRoper       H3'   C4'   C2'   O3'  !C3'
IMPRoper       H4'   C5'   C3'   O4'  !C4'
IMPRoper       H5'   O5'   H5''  C4'  !C5'



 END {CYT}

! ------------------------------------------------------------------------

RESIdue THY
 GROUp
  ATOM P     TYPE=P      CHARGE=1.20     END
  ATOM O1P   TYPE=O1P    CHARGE=-0.40    END
  ATOM O2P   TYPE=O2P    CHARGE=-0.40    END
  ATOM O5'   TYPE=O5R    CHARGE=-0.36    END
 GROUp
  ATOM C5'   TYPE=C5R    CHARGE=-0.070   END
  ATOM H5'   TYPE=H      CHARGE=0.035    END
  ATOM H5''  TYPE=H      CHARGE=0.035    END
 GROUp
  ATOM C4'   TYPE=C4R    CHARGE=0.065    END
  ATOM H4'   TYPE=H      CHARGE=0.035    END
  ATOM O4'   TYPE=O4R    CHARGE=-0.30    END
  ATOM C1'   TYPE=C1R    CHARGE=0.20     END
  ATOM H1'   TYPE=H      CHARGE=0.165    END

! Insert Base
 GROUp
  ATOM N1    TYPE=N1T    CHARGE=-0.19   EXCLUSION=( C4 )   END
  ATOM C6    TYPE=C6T    CHARGE=0.155   EXCLUSION=( N3 )   END
  ATOM H6    TYPE=H      CHARGE=0.035   END
 GROUp
  ATOM C2    TYPE=C2T    CHARGE=0.35    EXCLUSION=( C5 )   END
  ATOM O2    TYPE=ON     CHARGE=-0.35   END
 GROUp
  ATOM N3    TYPE=N3T    CHARGE=-0.26   END
  ATOM H3    TYPE=HN     CHARGE=0.26    END
 GROUp
  ATOM C4    TYPE=C4T    CHARGE=0.30    END
  ATOM O4    TYPE=ON     CHARGE=-0.30   END
 GROUp
  ATOM C5    TYPE=C5T    CHARGE=-0.035 END
  ATOM C7    TYPE=CC3E   CHARGE=-0.070 END ! name per IUPAC-IUB recomm.
  ATOM H71   TYPE=H      CHARGE=0.035   END ! name per IUPAC-IUB recomm.
  ATOM H72   TYPE=H      CHARGE=0.035   END ! name per IUPAC-IUB recomm.
  ATOM H73   TYPE=H      CHARGE=0.035   END ! name per IUPAC-IUB recomm.

 GROUp

! END
```

```
 GROUP
  ATOM C2'   TYPE=C2R    CHARGE=0.115     END
  ATOM H2'   TYPE=H      CHARGE=0.035     END
  ATOM O2'   TYPE=O2R    CHARGE=-0.40     END
  ATOM HO2'  TYPE=HO     CHARGE=0.25      END
 GROUP
  ATOM C3'   TYPE=C3R    CHARGE=-0.035    END
  ATOM H3'   TYPE=H      CHARGE=0.035     END

 GROUP
  ATOM O3'   TYPE=O3R    CHARGE=-0.36     END


 BOND P     O1P              BOND P     O2P              BOND P     O5'
 BOND O5'   C5'              BOND C5'   C4'              BOND C4'   O4'
 BOND C4'   C3'              BOND O4'   C1'              BOND C1'   N1
 BOND C1'   C2'              BOND N1    C2               BOND N1    C6
 BOND C2    O2               BOND C2    N3               BOND N3    H3
 BOND N3    C4               BOND C4    O4               BOND C4    C5
 BOND C5    C7               BOND C5    C6               BOND C2'   C3'
 BOND C3'   O3'              BOND C2'   O2'
 BOND O2'   HO2'
 BOND C5'   H5'              BOND C5'   H5''
 BOND C3'   H3'                   BOND C2'   H2'                BOND C1'   H1'
 BOND C4'   H4'              BOND C7    H71              BOND C7    H72
 BOND C7    H73              BOND C6    H6
{
 DIHEdral P    O5'   C5'   C4'              DIHEdral O5'   C5'   C4'   O4'
 DIHEdral O5'  C5'   C4'   C3'
}{
 DIHEdral C3'  C4'   O4'   C1'
 DIHEdral C4'  O4'   C1'   C2'              DIHEdral O4'   C1'   C2'   C3'

 DIHEdral C1'  C2'   C3'   C4'              DIHEdral O4'   C4'   C3'   O3'
 DIHEdral C5'  C4'   C3'   C2'              DIHEdral O2'   C2'   C3'   O3'
 DIHEdral O4'  C1'   N1    C2
 DIHEdral C3'  C2'   O2'   H2'


 ! New dihedrals
 DIHEdral  C5'  C4'  C3'  O3'              DIHEdral  C4'  O4'  C1'  N1
}

 IMPRoper O4    N3    C5    C4               IMPRoper C1'   C2    C6    N1
 IMPRoper O2    N1    N3    C2               IMPRoper C4    C5    C6    N1
 IMPRoper N1    C2    N3    C4               IMPRoper C2    N3    C4    C5
                                             IMPRoper N3    C4    C5    C6
 IMPRoper C5    C6    N1    C2               IMPRoper C6    N1    C2    N3
 IMPRoper H3    C2    C4    N3
 IMPRoper C7    C4    C6    C5               IMPRoper  H6   N1    C5    C6

!RIBOSE IMPROPERS
IMPRoper      H1'   C2'   O4'   N1 ! C1'
IMPRoper      H2'   C3'   C1'   O2' !C2'
```

```
IMPRoper      H3'   C4'   C2'   O3'  !C3'
IMPRoper      H4'   C5'   C3'   O4'  !C4'
IMPRoper      H5'   O5'   H5''  C4'  !C5'


 END {THY}


!-------------------------------------------------------------------------

RESIdue URI
GROUp
  ATOM P     TYPE=P     CHARGE=1.20    END
  ATOM O1P   TYPE=O1P   CHARGE=-0.40   END
  ATOM O2P   TYPE=O2P   CHARGE=-0.40   END
  ATOM O5'   TYPE=O5R   CHARGE=-0.36   END
 GROUp
  ATOM C5'   TYPE=C5R   CHARGE=-0.070  END
  ATOM H5'   TYPE=H     CHARGE=0.035   END
  ATOM H5''  TYPE=H     CHARGE=0.035   END
 GROUp
  ATOM C4'   TYPE=C4R   CHARGE=0.065   END
  ATOM H4'   TYPE=H     CHARGE=0.035   END
  ATOM O4'   TYPE=O4R   CHARGE=-0.30   END
  ATOM C1'   TYPE=C1R   CHARGE=0.165   END
  ATOM H1'   TYPE=H     CHARGE=0.035   END


 GROUp
  ATOM N1    TYPE=N1U   CHARGE=-0.19   EXCLUSION=( C4 )   END
  ATOM C6    TYPE=C6U   CHARGE=0.155   EXCLUSION=( N3 )   END
  ATOM H6    TYPE=H     CHARGE=0.035   END
 GROUp
  ATOM C2    TYPE=C2U   CHARGE=0.30    EXCLUSION=( C5 )   END
  ATOM O2    TYPE=ON    CHARGE=-0.30   END
 GROUp
  ATOM N3    TYPE=N3U   CHARGE=-0.28   END
  ATOM H3    TYPE=HN    CHARGE=0.26    END
 GROUp
  ATOM C4    TYPE=C4U   CHARGE=0.28    END
  ATOM O4    TYPE=ON    CHARGE=-0.30   END
 GROUp
  ATOM C5    TYPE=C5U   CHARGE=-0.035  END  !JPR
  ATOM H5    TYPE=H     CHARGE=0.035   END  !JPR

 GROUP
  ATOM C2'   TYPE=C2R   CHARGE=0.115   END
  ATOM H2'   TYPE=H     CHARGE=0.035   END  !
  ATOM O2'   TYPE=O2R   CHARGE=-0.40   END
  ATOM HO2'    TYPE=HO    CHARGE=0.25   END
 GROUP
  ATOM C3'   TYPE=C3R   CHARGE=-0.035  END
  ATOM H3'   TYPE=H     CHARGE=0.035   END
 GROUP
  ATOM O3'   TYPE=O3R   CHARGE=-0.36   END
```

```
 BOND P    O1P                 BOND P    O2P                 BOND P    O5'
 BOND O5'  C5'                 BOND C5'  C4'                 BOND C4'  O4'
 BOND C4'  C3'                 BOND O4'  C1'                 BOND C1'  N1
 BOND C1'  C2'                 BOND N1   C2                  BOND N1   C6
 BOND C2   O2                  BOND C2   N3                  BOND N3   H3
 BOND N3   C4                  BOND C4   O4                  BOND C4   C5

 BOND C5   C6                  BOND C2'  C3'                 BOND C3'  O3'
 BOND C2'  O2'
 BOND C5   H5                  BOND C6   H6

 BOND O2'  HO2'
 BOND C5'  H5'          BOND C5'  H5''          BOND C4'  H4'
 BOND C3'  H3'          BOND C2'  H2'           BOND C1'  H1'
{
 DIHEdral P   O5'  C5'  C4'              DIHEdral O5'  C5'  C4'  O4'
 DIHEdral O5'  C5'  C4'  C3'
}{
 DIHEdral C3'  C4'  O4'  C1'
 DIHEdral C4'  O4'  C1'  C2'             DIHEdral O4'  C1'  C2'  C3'

 DIHEdral C1'  C2'  C3'  C4'             DIHEdral O4'  C4'  C3'  O3'
 DIHEdral C5'  C4'  C3'  C2'             DIHEdral O2'  C2'  C3'  O3'
 DIHEdral O4'  C1'  N1   C2
 DIHEdral C3'  C2'  O2'  H2'

 DIHEdral P    O3'  C3'  C2'             DIHEdral P    O3'  C3'  C4'
 ! New dihedrals
 DIHEdral  C5'  C4'  C3'  O3'            DIHEdral  C4'  O4'  C1'  N1
}
                                            IMPRoper C1'  C2   C6   N1
 IMPRoper O2   N1   N3   C2                 IMPRoper H3   C2   C4   N3
 IMPRoper O4   N3   C5   C4                 IMPRoper N1   C2   N3   C4
 IMPRoper C2   N3   C4   C5                 IMPRoper N3   C4   C5   C6
 IMPRoper C4   C5   C6   N1                 IMPRoper C5   C6   N1   C2
 IMPRoper C6   N1   C2   N3
 IMPRoper H5   C4   C6   C5                 IMPRoper H6   N1   C5   C6

 !GENERAL RIBOSE IMPROPERS
IMPRoper      H1'  C2'  O4'  N1  !C1'
IMPRoper      H2'  C3'  C1'  O2' !C2'
IMPRoper      H3'  C4'  C2'  O3' !C3'
IMPRoper      H4'  C5'  C3'  O4' !C4'
IMPRoper      H5'  O5'  H5'' C4' !C5'



END {URI}

!-------------------------------------------------------------------

PRESidue DEOX     ! Patch to make DEOXYribose of the ribose
 DELETE ATOM O2'   END
```

```
 DELETE ATOM HO2'    END
GROUP
 MODIFY  ATOM C2'  TYPE=C2D   CHARGE=-0.07    END
 MODIFY  ATOM C5'  TYPE=C5D   CHARGE=-0.07    END
 MODIFY  ATOM C4'  TYPE=C4D   CHARGE=0.065    END
 MODIFY  ATOM O4'  TYPE=O4D   CHARGE=-0.30    END
 MODIFY  ATOM C1'  TYPE=C1D   CHARGE=0.165    END
 MODIFY  ATOM C3'  TYPE=C3D   CHARGE=-0.035   END
 ADD     ATOM H2'' TYPE=H     CHARGE=0.035    END

 ADD BOND   C2'  H2''
 ADD ANGLE  C1'  C2'  H2'
 ADD ANGLE  C3'  C2'  H2''
 ADD ANGLE  H2'  C2'  H2''
 ADD IMPRoper   H2'  C3'  H2'' C1'! C2' chirality term
END {DEOX}

!-------------------------------------------------------------------------


PRESidue 3TER                ! 3-terminus (without phosphate)
                             ! should be used as "LAST 3TER  HEAD - * END"
 GROUp                       ! i.e. to be patched to the last RNA residue
  MODIFY ATOM -C3'    TYPE=C3R    CHARGE=0.15    END
  MODIFY ATOM -O3'    TYPE=OH     CHARGE=-0.40   END
  ADD ATOM -H3T  TYPE=HO      CHARGE=0.25         END
 !
 ADD BOND -O3'  -H3T
 ADD ANGLe -C3'  -O3'  -H3T
! ADD DIHEdral -C4'  -C3'  -O3'  -H3T
END {3TER}



! ------------------------------------------------------------------------
PRESidue 5TER          ! 5-terminus (without phosphate)
 !                     ! should be used as "FIRST  5TER  TAIL + * END"
 GROUp                 ! i.e. to be patched to the first RNA residue
  ADD ATOM +H5T  TYPE=HO    CHARGE=0.25    END
  MODIFY ATOM +O5'  TYPE=OH  CHARGE=-0.40  END
  MODIFY ATOM +C5'  TYPE=C5R  CHARGE=0.15  END
  DELETE ATOM +P   END
  DELETE ATOM +O1P END
  DELETE ATOM +O2P END
 !
 ADD BOND +H5T   +O5'
 ADD ANGLe +H5T   +O5'  +C5'
! ADD DIHEdral +H5T   +O5'  +C5'  +C4'
END {5TER}

!-------------------------------------------------------------------------

PRESidue NUC          ! patch for nucleic acid backbone
                      ! should be used as "LINK NUC  HEAD - *  TAIL + *  END"
                      ! i.e. it links the previous RNA residue (-) with
                      ! the current one (+)
```

```
    GROUp

     MODIFY ATOM -O3' END    !
     MODIFY ATOM +P    END   !
     MODIFY ATOM +O1P END    ! this should correctly define the electrostatic

     MODIFY ATOM +O2P END    ! group boundary

     MODIFY ATOM +O5' END    !
    ADD BOND -O3' +P
    ADD ANGLE -C3' -O3' +P
    ADD ANGLE -O3' +P  +O1P
    ADD ANGLE -O3' +P  +O2P
    ADD ANGLE -O3' +P  +O5'
   !ADD DIHEdral -O3' +P  +O5'  +C5'

   ! ADD DIHEdral -C4' -C3' -O3'  +P
   ! ADD DIHEdral -C3' -O3' +P   +O5'

   END {NUC}

   !-------------------------------------------------------------------------

   ! mod by anda-----------------------------------------------------------------------------------
   PRESidue 2AP

   GROUP
    DELETE ATOM H2 END
    ADD ATOM N2   TYPE=N2G   CHARGE=-0.91   END
    ADD ATOM H21  TYPE=H2    CHARGE=0.39    END
    ADD ATOM H22  TYPE=H2    CHARGE=0.38    END

    MODIFY ATOM N1 CHARGE=-0.72 END
    MODIFY ATOM C2 CHARGE= 0.98 END
    MODIFY ATOM N3 CHARGE=-0.80 END
    MODIFY ATOM C4 CHARGE= 0.60 END
    MODIFY ATOM C5 CHARGE= 0.08 END
    MODIFY ATOM C6 CHARGE= 0.20 END
    MODIFY ATOM N7 CHARGE=-0.45 END !changed from -0.61 to -0.45 for neutrality
    MODIFY ATOM C8 CHARGE= 0.27 END
    MODIFY ATOM N9 CHARGE=-0.25 END
!     MODIFY ATOM N2 CHARGE=-0.91 END
!     MODIFY ATOM H21 CHARGE= 0.39 END
!     MODIFY ATOM H22 CHARGE= 0.38 END
    MODIFY ATOM H6 CHARGE= 0.12 END
    MODIFY ATOM H8 CHARGE= 0.11 END

    ADD BOND C2   N2
    ADD BOND N2   H21
    ADD BOND N2   H22

    ADD ANGLE N1 C2 N2
    ADD ANGLE C2 N2 H21
    ADD ANGLE C2 N2 H22
    ADD ANGLE H21 N2 H22
    ADD ANGLE N3 C2 N2
```

```
 ADD IMPRoper N3    C2    N2    H21
 ADD IMPRoper N2    N3    N1    C2
 ADD IMPRoper H22   H21   C2    N2
 END {2AP}
! mod by anda-------------------------------------------------------------------------


set echo=true end
```

.2 Xplor-NIH calculation input files

Since all calculations were carried out under the same conditions, only the input files for the 13mer2AP calculation is shown. The input scripts are based on the example files of the XPLOR-NIH package (refine_full.py and sa.inp) but were substantially modified in the course of this work. The first script was used to calculate the starting structure fir the second script.

```
   language
remarks file  nmr/sa.inp
remarks  Simulated annealing protocol for NMR structure determination.
remarks  The starting structure for this protocol can be any structure with
remarks  a reasonable geometry, such as randomly assigned torsion angles or
remarks  extended strands.
remarks  Author: Michael Nilges

{====>}
evaluate ($init_t = 3000)       {*Initial simulated annealing temperature.*}
{====>}
evaluate ($high_steps= 48000 )         {*Total number of steps at high temp.*}
{====>}
evaluate ($cool_steps = 6000 )      {*Total number of steps during cooling.*}

parameter                                          {*Read the parameter file.*}
{====>}
   @13mer_2AP_c_dna.param
end

{====>}
structure @13mer_2AP_c_dna.psf end                      {*Read the structure file.*}
{====>}
coordinates @13mer_2AP_c_dna.pdb                   {*Read the coordinates.*}


noe
{====>}
   nres=3000             {*Estimate greater than the actual number of NOEs.*}
   class all
{====>}
   @13mer2AP_xplor_all_3.tbl                            {*Read NOE distance ranges.*}
   @hbond_13mer_2AP.tbl
end

{====>}
restraints dihedral
   nass = 1000
   @dihedral_13mer_2AP_BDNA.tbl                        {*Read dihedral angle
   restraints.*}
end
@plane_13mer_2AP.inp

{* Reduce the scaling factor on the force applied to disulfide           *}
```

```
{* bonds and angles from 1000.0 to 100.0 in order to reduce computation instability. *}

parameter
      bonds ( name SG ) ( name SG ) 100. TOKEN
      angle ( name CB ) ( name SG ) ( name SG ) 50. TOKEN
end


flags exclude * include bonds angle impr vdw elec noe cdih plan end

                        {*Friction coefficient for MD heatbath, in 1/ps.   *}
vector do (fbeta=10) (all)
                        {*Uniform heavy masses to speed molecular dynamics.*}
vector do (mass=100) (all)

noe                             {*Parameters for NOE effective energy term.*}
  ceiling=1000
  averaging  * cent
  potential  * soft
  scale      * 50.
  sqoffset   * 0.0
  sqconstant * 1.0
  sqexponent * 2
  soexponent * 1
  asymptote  * 0.1                          {*Initial value--modified later.*}
  rswitch    * 0.5
end

parameter                       {*Parameters for the repulsive energy term.*}
    nbonds
      repel=1.                   {*Initial value for repel--modified later.*}
      rexp=2 irexp=2 rcon=1.
      nbxmod=3
      wmin=0.01
      cutnb=4.5 ctonnb=2.99 ctofnb=3.
      tolerance=0.5
   end
end

restraints dihedral
      scale=5.
end

{====>}
evaluate ($end_count=100)          {*Loop through a family of 100 structures.*}

coor copy end

evaluate ($count = 0)
evaluate ($count2 = 0)
while ($count < $end_count ) loop main

   evaluate ($count=$count+1)
   evaluate ($count2=$count2+1)
```

```
  coor swap end
  coor copy end

    {* ================================================ Initial minimization.*}
 restraints dihedral   scale=5.   end
  noe asymptote * 0.1  end
  parameter  nbonds repel=1.   end end
  constraints interaction
          (all) (all) weights * 1  vdw 0.002 end end
  minimize powell nstep=50 drop=10.  nprint=25 end


    {* ============================================ High-temperature dynamics.*}
  constraints interaction (all) (all)
              weights * 1  angl 0.4  impr 0.1 vdw 0.002 end end

  evaluate ($nstep1=int($high_steps * 2. / 3. ) )
  evaluate ($nstep2=int($high_steps * 1. / 3. ) )

  dynamics  verlet
     nstep=$nstep1   timestep=0.003   iasvel=maxwell   firstt=$init_t
     tcoupling=true  tbath=$init_t  nprint=50  iprfrq=0
  end


    {* ============== Tilt the asymptote and increase weights on geometry.*}
  noe asymptote * 1.0  end

  constraints interaction
            (all) (all) weights * 1  vdw 0.002  end end

  {* Bring scaling factor for S-S bonds back *}

  parameter
    bonds ( name SG ) ( name SG ) 1000. TOKEN
    angle ( name CB ) ( name SG ) ( name SG ) 500. TOKEN
 end

  dynamics  verlet
     nstep=$nstep2   timestep=0.001    iasvel=current   tcoupling=true
     tbath=$init_t  nprint=50  iprfrq=0
  end

   {* ================================================  Cool the system.*}

 restraints dihedral   scale=200.   end

  evaluate ($final_t =  25)      { K }
  evaluate ($tempstep = 25)      { K }

  evaluate ($ncycle = ($init_t-$final_t)/$tempstep)
  evaluate ($nstep = int($cool_steps/$ncycle))

  evaluate ($ini_rad  = 0.9)        evaluate ($fin_rad  = 0.75)
  evaluate ($ini_con=  0.003)       evaluate ($fin_con=  4.0)
```

```
   evaluate ($bath  = $init_t)
   evaluate ($k_vdw = $ini_con)
   evaluate ($k_vdwfact = ($fin_con/$ini_con)^(1/$ncycle))
   evaluate ($radius=    $ini_rad)
   evaluate ($radfact = ($fin_rad/$ini_rad)^(1/$ncycle))

   evaluate ($i_cool = 0)
   while ($i_cool < $ncycle) loop cool
      evaluate ($i_cool=$i_cool+1)

      evaluate ($bath  = $bath  - $tempstep)
      evaluate ($k_vdw=min($fin_con,$k_vdw*$k_vdwfact))
      evaluate ($radius=max($fin_rad,$radius*$radfact))

      parameter  nbonds repel=$radius   end end
      constraints interaction (all) (all)
                    weights * 1. vdw $k_vdw end end

      dynamics  verlet
         nstep=$nstep time=0.001 iasvel=current firstt=$bath
         tcoup=true tbath=$bath nprint=$nstep iprfrq=0
      end


{====>}                                                {*Abort condition.*}
      evaluate ($critical=$temp/$bath)
      if ($critical >  10. ) then
         display  ****&&&&& rerun job with smaller timestep (i.e., 0.003)
         stop
      end if

   end loop cool
   {* ================================================ Final minimization.*}

   constraints interaction (all) (all) weights * 1. vdw 1. end end

   parameter                          {*Parameters for the repulsive energy term.*}
     nbonds
      repel=0.                      {*Initial value for repel--modified later.*}
      SWITCH
      VSWITCH
      RDIE
      cutnb=11.5
      nbxmod=5
      wmin=0.01
      ctofnb=10.5
      ctonnb=9.5
      tolerance=0.5
    end
   end
   flags exclude * include bonds angle impr vdw elec noe cdih plan end

   minimize powell nstep=3000 drop=10.0 nprint=25 end

   {* ================================== Write out the final structure(s).*}
   print threshold=0.5 noe
```

```
    evaluate ($rms_noe=$result)
    evaluate ($violations_noe=$violations)
    print threshold=0.0 noe
    evaluate ($rms_noe22=$result)
    print threshold=5. cdih
    evaluate ($rms_cdih=$result)
    evaluate ($violations_cdih=$violations)
    print thres=0.05 bonds
    evaluate ($rms_bonds=$result)
    print thres=5. angles
    evaluate ($rms_angles=$result)
    print thres=5. impropers
    evaluate ($rms_impropers=$result)
    remarks ============================================================
    remarks             overall,bonds,angles,improper,vdw,noe,cdih,elec
    remarks energies: $ener, $bond, $angl, $impr, $vdw, $noe, $cdih, $elec
    remarks ============================================================
    remarks             bonds,angles,impropers,noe,cdih
    remarks rms-d: $rms_bonds,$rms_angles,$rms_impropers,$rms_noe,$rms_cdih
    remarks ============================================================
    remarks                noe,  cdih
    remarks violations.: $violations_noe, $violations_cdih
    remarks ============================================================
    remarks enviol: $ener $$violations_noe $violations_cdih
    remarks ============================================================


{=====>}                         {*Name(s) of the family of final structures.*}
    evaluate ($filename="z13mer_2AP_c_dna_test"+encode($count)+".pdb")

    write coordinates output =$filename end

    evaluate ($filename2="z13mer_2AP_c_dna_test"+encode($count2)+".noe")
    set display=$filename2 end
    @@picktbl_13mer2AP_all
    close $filename2 end
    set display=OUTPUT end

end loop main

stop



seed                    = 10
numberOfStructures      = 100
startStructure          = 1



# User-specific which has to be adjusted for each new sample
andaSampleName  = "13mer_2AP"                                   # specify sample name
andaNumResAll   = 26                                            # Total number of
     Residues
andaDiheIdeal   = "B"                                           # specify which
     dihedral constraint set should be used
```

```
andaDiheBound   = 30.0                                                    # specify upper and
    lower bound for dihedral constraints
andaDiheBound2  = 30.0                                                    # specify loose upper
    and lower bound for dihedral constraints
andaSeqStrand1  = "GUA CYT THY GUA CYT ADE ADE ADE CYT GUA THY CYT GUA" # Sequence of Strand I
andaSeqStrand2  = "CYT GUA ADE CYT GUA THY THY THY GUA CYT ADE GUA CYT" # Sequence of Strand II
outFilename     = andaSampleName+"_STRUCTURE.pdb"                         # pdb output filename
andaNOEexp      = "NOE_13mer2AP_xplor_ohneH5_clean.tbl"                  # file for reading in
    experimental NOE constraints
andaDipoInp     = "RDC_13mer2AP_safe.inp.xplor"                          # file for reading in
    experimental RDC constraints
andaDipoInpMe   = "RDC_13mer2AP_safe_Me.inp.xplor"                          # file for reading
    in experimental Me-RDC constraints

# User-specific data, which can be adjusted for external script,
# but which can also be generated from the information given above
andaNumRes      = andaNumResAll/2
andaInitCoord   = "start_"+andaSampleName+".pdb"                # file created with initial
    extended structure coordinates
andaInitPSF     = "start_"+andaSampleName+".psf"                # file created with initial
    extended structure
andaPlan        = "plane_13mer_2AP.inp"                         # file for reading in planar
    constraints
andaOrie        = "dna_positional_anda.setup"                   # file for reading in ORIE
    constraints
andaHbond       = "hbond_13mer_2AP.tbl"                         # file for reading in Hbond
    constraints
andaDihe        = None                                          # file for reading in ideal
    dihedral constraints
andaRAMA        = "nucleic"                                     # file for reading in ideal
    RAMA constraints



# Store each residue in list andaRes!
# (Index+1) corresponds to residue number
andaRes = range(andaNumResAll)
andai   = 0
andaii  = 3
for andabla in range(andaNumRes):
  andaRes[andabla]=andaSeqStrand1[andai:andaii]
  andaRes[andabla+andaNumRes]=andaSeqStrand2[andai:andaii]
  andai  = andai+4
  andaii = andaii+4


if andaDihe==None:
    # Generate dihedral input table with ideal values for A- or B-DNA
    # input values for dihedrals
    alphaA,alphaB   = -50.0,-46.0
    betaA,betaB     = 172.0, -147.0
    gammaA,gammaB   = 41.0,36.0
    deltaA,deltaB   = 79.0,157.0
    epsA,epsB       = -146.0,155.0
    zetaA,zetaB     = -78.0,-96.0
```

```
fileHandle = open (andaSampleName+'_dihe.tbl','w')
#
# B-DNA conformation
#
if andaDiheIdeal=="B":
# Alpha angle
  fileHandle.write ("! Ideal dihedral values for B-DNA\n! Values taken from Roberts: NMR
      of Macromolecules\n\n\n!Alpha dihedral\n\n")
  for andai in range(1,andaNumRes):
        fileHandle.write ("assign ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )\n       ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )   1.0 %s %s 2\n\n" %
            (andai,andai+1,andai+1,andai+1,alphaB,andaDiheBound))
  for andai in range(14,andaNumRes+13):
        fileHandle.write ("assign ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )\n       ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )   1.0 %s %s 2\n\n" %
            (andai,andai+1,andai+1,andai+1,alphaB,andaDiheBound))
# Beta angle
  fileHandle.write ("\n!Beta dihedral\n\n")
  for andai in range(2,andaNumRes+1):
        fileHandle.write ("assign ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )\n       ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,betaB,andaDiheBound))
  for andai in range(15,andaNumRes+14):
        fileHandle.write ("assign ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )\n       ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,betaB,andaDiheBound))
# Gamma angle
  fileHandle.write ("\n!Gamma dihedral\n\n")
  for andai in range(1,andaNumRes+1):
        fileHandle.write ("assign ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )\n       ( resid %s   and name C4'  )\n       ( resid %s   and
            name C3'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,gammaB,andaDiheBound))
  for andai in range(14,andaNumRes+14):
        fileHandle.write ("assign ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )\n       ( resid %s   and name C4'  )\n       ( resid %s   and
            name C3'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,gammaB,andaDiheBound))
# Delta angle
  fileHandle.write ("\n!Delta dihedral\n\n")
  for andai in range(1,andaNumRes+1):
        fileHandle.write ("assign ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )\n       ( resid %s   and name C3'  )\n       ( resid %s   and
            name O3'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,deltaB,andaDiheBound))
  for andai in range(14,andaNumRes+14):
        fileHandle.write ("assign ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )\n       ( resid %s   and name C3'  )\n       ( resid %s   and
            name O3'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,deltaB,andaDiheBound))
# Epsilon angle, loose bound
  fileHandle.write ("\n!Epsilon dihedral\n\n")
```

```
  for andai in range(2,andaNumRes):
        fileHandle.write ("assign ( resid %s   and name C4'  )\n       ( resid %s   and
            name C3'  )\n       ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai+1,epsB,andaDiheBound2))
  for andai in range(15,andaNumRes+13):
        fileHandle.write ("assign ( resid %s   and name C4'  )\n       ( resid %s   and
            name C3'  )\n       ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai+1,epsB,andaDiheBound2))
# Zeta angle, loose bound
  fileHandle.write ("\n!Zeta dihedral\n\n")
  for andai in range(2,andaNumRes):
        fileHandle.write ("assign ( resid %s   and name C3'  )\n       ( resid %s   and
            name O3'  )\n       ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai+1,andai+1,zetaB,andaDiheBound2))
  for andai in range(15,andaNumRes+13):
        fileHandle.write ("assign ( resid %s   and name C3'  )\n       ( resid %s   and
            name O3'  )\n       ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai+1,andai+1,zetaB,andaDiheBound2))
#
# A-BDNA conformation
#
elif andaDiheIdeal=="A":
# Alpha angle
  fileHandle.write ("! Ideal dihedral values for A-DNA\nValues taken from Roberts: NMR of
      Macromolecules\n\n\n!Alpha dihedral\n\n")
  for andai in range(1,andaNumRes):
        fileHandle.write ("assign ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )\n       ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )   1.0 %s %s 2\n\n" %
            (andai,andai+1,andai+1,andai+1,alphaA,andaDiheBound))
  for andai in range(14,andaNumRes+13):
        fileHandle.write ("assign ( resid %s   and name O3'  )\n       ( resid %s   and
            name P    )\n       ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )   1.0 %s %s 2\n\n" %
            (andai,andai+1,andai+1,andai+1,alphaA,andaDiheBound))
# Beta angle
  fileHandle.write ("\n!Beta dihedral\n\n")
  for andai in range(2,andaNumRes+1):
        fileHandle.write ("assign ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )\n       ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,betaA,andaDiheBound))
  for andai in range(15,andaNumRes+14):
        fileHandle.write ("assign ( resid %s   and name P    )\n       ( resid %s   and
            name O5'  )\n       ( resid %s   and name C5'  )\n       ( resid %s   and
            name C4'  )   1.0 %s %s 2\n\n" %
            (andai,andai,andai,andai,betaA,andaDiheBound))
# Gamma angle
  fileHandle.write ("\n!Gamma dihedral\n\n")
  for andai in range(1,andaNumRes+1):
        fileHandle.write ("assign ( resid %s   and name O5'  )\n       ( resid %s   and
            name C5'  )\n       ( resid %s   and name C4'  )\n       ( resid %s   and
```

```
                name C3' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai,gammaA,andaDiheBound))
    for andai in range(14,andaNumRes+14):
          fileHandle.write ("assign ( resid %s   and name O5' )\n       ( resid %s   and
                name C5' )\n       ( resid %s   and name C4' )\n       ( resid %s   and
                name C3' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai,gammaA,andaDiheBound))
  # Delta angle
    fileHandle.write ("\n!Delta dihedral\n\n")
    for andai in range(1,andaNumRes+1):
          fileHandle.write ("assign ( resid %s   and name C5' )\n       ( resid %s   and
                name C4' )\n       ( resid %s   and name C3' )\n       ( resid %s   and
                name O3' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai,deltaA,andaDiheBound))
    for andai in range(14,andaNumRes+14):
          fileHandle.write ("assign ( resid %s   and name C5' )\n       ( resid %s   and
                name C4' )\n       ( resid %s   and name C3' )\n       ( resid %s   and
                name O3' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai,deltaA,andaDiheBound))
  # Epsilon angle, loose bound
    fileHandle.write ("\n!Epsilon dihedral\n\n")
    for andai in range(2,andaNumRes):
          fileHandle.write ("assign ( resid %s   and name C4' )\n       ( resid %s   and
                name C3' )\n       ( resid %s   and name O3' )\n       ( resid %s   and
                name P   )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai+1,epsA,andaDiheBound2))
    for andai in range(15,andaNumRes+13):
          fileHandle.write ("assign ( resid %s   and name C4' )\n       ( resid %s   and
                name C3' )\n       ( resid %s   and name O3' )\n       ( resid %s   and
                name P   )   1.0 %s %s 2\n\n" %
                (andai,andai,andai,andai+1,epsA,andaDiheBound2))
  # Zeta angle, loose bound
    fileHandle.write ("\n!Zeta dihedral\n\n")
    for andai in range(2,andaNumRes):
          fileHandle.write ("assign ( resid %s   and name C3' )\n       ( resid %s   and
                name O3' )\n       ( resid %s   and name P   )\n       ( resid %s   and
                name O5' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai+1,andai+1,zetaA,andaDiheBound2))
    for andai in range(15,andaNumRes+13):
          fileHandle.write ("assign ( resid %s   and name C3' )\n       ( resid %s   and
                name O3' )\n       ( resid %s   and name P   )\n       ( resid %s   and
                name O5' )   1.0 %s %s 2\n\n" %
                (andai,andai,andai+1,andai+1,zetaA,andaDiheBound2))
  fileHandle.close()
  andaDihe=andaSampleName+'_dihe.tbl'


################################################## end of anda mod
    ######################################################################

xplor.parseArguments()                  # check for typos on the command-line

simWorld.setRandomSeed(seed)

#
# Create the PSF and initial PDB files as an extended structure
```

```
# mod by anda
#
import protocol
protocol.initParams("anda_old_nucleic")
protocol.initTopology("anda_old_nucleic")
#import psfGen
#from psfGen import pdbToPSF
#pdbToPSF(andaInitCoord,psfFilename=andaInitPSF,andaPar="anda_old_nucleic",customRename=0)
#from psfGen import seqToPSF
## generates PSF-information from Sequence
#seqToPSF(andaSeqStrand1,seqType='dna',customRename=1)
#pass
#seqToPSF(andaSeqStrand2,startResid=andaNumRes+1,seqType='dna',customRename=1)
#pass
## writes out PSF info to file
#xplor.command("write psf output=%s end" % andaInitPSF)
## generates an extended structure
#protocol.genExtendedStructure(andaInitCoord,numerator=10,verbose=1,maxFixupIters=2000)
protocol.initStruct(andaInitPSF)


#
# starting coords
#
protocol.initCoords(andaInitCoord)

# protocol.fixupCovalentGeom(verbose=1)
# list of potential terms used in refinement
from potList import PotList
potList = PotList()
crossTerms=PotList('cross terms') # can add some pot terms which are not
                                  # refined against- but included in analysis


# parameters to ramp up during the simulated annealing protocol
#
from simulationTools import MultRamp, StaticRamp, InitialParams
rampedParams=[]
highTempParams=[]

from varTensorTools import create_VarTensor, calcTensor
media={}
for medium in ['pf1']:
    media[medium] = create_VarTensor(medium)
    pass


from xplorPot import XplorPot

#planarity restraints
xplor.command("@%s" % andaPlan)
potList.append(XplorPot("plan",xplor.simulation))



#initialize the aa-aa positional database
```

```
#xplor.command("@%s" % andaOrie)
#potList.append(XplorPot("orie"))

#NOE potentials
from noePotTools import create_NOEPot
noePots = PotList("noe")
noe = create_NOEPot("noeAll",andaNOEexp)
noe.setPotType("hard")
noePots.append(noe)

# need to be satisfied by all structures
noeHB  = create_NOEPot("noeNH",andaHbond)
noeHB.setPotType("hard")
noeHB.setScale(1000)
noePots.append(noeHB)
potList.append(noePots)
rampedParams.append( StaticRamp("noePots.setScale( 50 )") )

protocol.initDihedrals(andaDihe)
potList.append(XplorPot("CDIH"))
highTempParams.append( StaticRamp("potList['CDIH'].setScale(200)") )
rampedParams.append( StaticRamp("potList['CDIH'].setScale(200)") )
#rampedParams.append( MultRamp(10,200,"potList['CDIH'].setScale(VALUE)") )

#protocol.initRamaDatabase(andaRAMA)
#potList.append(XplorPot("rama"))
#highTempParams.append( StaticRamp("potList['RAMA'].setScale(0)") )
#rampedParams.append( MultRamp(1,1.0,"potList['RAMA'].setScale(VALUE)") )

from rdcPotTools import Da_prefactor, create_RDCPot, scale_toCH

#from csaPotTools import create_CSAPot
#csaPots = PotList("csa")
#for (name,medium,force,tbl) in [("POP", 'phg3',1,"justin_csa.tbl"),
#                                ("POP2",'bic2',1,"justin_csa_bcl.tbl")]:
#    term = create_CSAPot(name,oTensor=media[medium],file=tbl)
#    term.setDaScale( -term.DaScale() ) #switch sign
#    term.setScale(force)
#    csaPots.append( term )
#    pass
#
#potList.append(csaPots)
#rampedParams.append( MultRamp(0.01,0.2,"csaPots.setScale( VALUE )") )
#
#
## Rh same for jch2 and phos/pho2, but da is different
## let's add this later with a cosRatio2 potential term
#
rdcPots = PotList('rdcs')
# weight is the relative weighting of expts, as determined by expt. error
for (name,medium,weight,files) in [
    ('JCH' ,'pf1',5    ,andaDipoInp),('methyl' ,'pf1',0.5 ,andaDipoInpMe)
    ]:
    term = create_RDCPot(name,oTensor=media[medium],defThreshold=1.9)
    if type(files)==type('string'):
        files=(files,)
```

```
        pass
    for file in files:
        term.addRestraints( open(file).read() )
        pass
    term.setShowAllRestraints(1)
    term.setScale(weight)
    #term.setAveType("average")
    term.setAveType("sum")
    print name
    scale_toCH(term) #also sets useDistance
    print term.info()
    print term.gyroA()
    rdcPots.append(term)
    pass


## proton setup
#for key in ('HABS', 'HAB2'): rdcPots[key].setUseSign(0)
#
#for key in ('HABS', 'HSIG', 'HAB2', 'HSI2'):
#    rdcPots[key].setPotType('square');
#    rdcPots[key].setAveType('sum')
#    pass

potList.append(rdcPots)
rampedParams.append( MultRamp(0.01,1,"rdcPots.setScale( VALUE )") )


from rdcPotTools import Da_prefactor

print "factor:", Da_prefactor['CH'] / Da_prefactor["NH"]
for medium in media.values():
    calcTensor(medium)
    print "medium: ", medium.instanceName(), \
          "Da: ",medium.Da(), "Rh: ",medium.Rh()
    pass

#let's try fixing Da, Rh:
print medium
for (medium,Da,Rh) in (('pf1',-41.02,0.321),):
    medium = media[medium]
    medium.setDa(Da)
    medium.setRh(Rh)
    pass


## set up J coupling
#from jCoupPotTools import create_JCoupPot
#jCoup = create_JCoupPot("jcoup","couplings.tbl",
#                        A=15.3,B=-6.1,C=1.6,phase=0 )
#jCoup.setThreshold(0)
#jCoup.setScale(10)
#potList.append(jCoup)}

#protocol.initNBond(cutnb=4.5)
potList.append( XplorPot("VDW") )
```

```
potList.append( XplorPot("elec") )
#rampedParams.append( MultRamp(0.99,0.78,
#                               "xplor.command('param nbonds repel VALUE end end')") )
#rampedParams.append( MultRamp(.0001,4,
#                               "xplor.command('param nbonds rcon VALUE end end')") )
rampedParams.append( StaticRamp("""xplor.command('''param nbonds
                                                    atom
                                                    repel=0
                                                    wmin=0.01
                                                    nbxmod=5
                                                    cutnb=58.5
                                                    ctonnb=56.5
                                                    ctofnb=57.5
                                                    tolerance=0.5
                                                    rdie
                                                    vswitch
                                                    switch
                                    end end''')""") )
#highTempParams.append( StaticRamp("""protocol.initNBond(cutnb=100,
#                                                       tolerance=45,
#                                                       #repel=1.2,
#                                                       #selStr='name P'
#                                                       )""") )

for name in ("bond","angl","impr"):
    potList.append( XplorPot(name) )
    pass
rampedParams.append( MultRamp(0.4,1.0,"potList['ANGL'].setScale(VALUE)"))
rampedParams.append( MultRamp(0.1,1.0,"potList['IMPR'].setScale(VALUE)"))


from ivm import IVM
import varTensorTools
mini = IVM()              #initial alignment of orientation tensor axes

for medium in (('pf1'),): media[medium].setFreedom("fixDa, fixRh")
#for medium in ('bic2',):
#    media[medium].setFreedom("fixDa, fixRh, fixAxisTo bic1")
#for medium in ('phg2','phg3',):
#    media[medium].setFreedom("fixDa, fixRh, fixAxisTo phg1")
varTensorTools.topologySetup(mini,media.values())

protocol.initMinimize(mini,
                      numSteps=20)
mini.fix("not resname ANI")
mini.run()                #this initial minimization is not strictly necessary

#uncomment to allow Da, Rh to vary
for medium in (('pf1'),): media[medium].setFreedom("varyDa, varyRh")
#for medium in ('bic2',):
#    media[medium].setFreedom("varyDa, varyRh, fixAxisTo bic1")
#for medium in ('phg2','phg3',):
#    media[medium].setFreedom("varyDa, fixAxisTo phg1, fixRhTo phg1")

dyn = IVM()
protocol.initDynamics(dyn,potList=potList)
```

```
varTensorTools.topologySetup(dyn,media.values())
protocol.torsionTopology(dyn)

#
#
# Give atoms uniform weights, except for the anisotropy axis
from atomAction import SetProperty
AtomSel("not resname ANI").apply( SetProperty("mass",100.) )
varTensorTools.massSetup(media.values(),300)
AtomSel("all            ").apply( SetProperty("fric",10.) )


##
## minc used for final cartesian minimization
##
from selectTools import IVM_groupRigidSidechain
minc = IVM()
protocol.initMinimize(minc,potList=potList)
IVM_groupRigidSidechain(minc)
protocol.cartesianTopology(minc,"not resname ANI")
varTensorTools.topologySetup(minc,media.values())

init_t1 = 200000
init_t2 = 20000
init_t3 = 3000

from simulationTools import AnnealIVM
anneal1= AnnealIVM(initTemp =init_t1,
                   finalTemp=init_t2,
                   tempStep =5000,
                   ivm=dyn,
                   rampedParams = rampedParams)
anneal2= AnnealIVM(initTemp =init_t2,
                   finalTemp=init_t3,
                   tempStep =500,
                   ivm=dyn,
                   rampedParams = rampedParams)
anneal3= AnnealIVM(initTemp =init_t3,
                   finalTemp=25,
                   tempStep =25,
                   ivm=dyn,
                   rampedParams = rampedParams)


# initialize parameters for initial minimization.
InitialParams( rampedParams )
# high-temp dynamics setup - only need to specify parameters which
#   differfrom initial values in rampedParams
InitialParams( highTempParams )

# initial minimization
protocol.initMinimize(dyn,
                      potList=[potList['CDIH'],potList['IMPR']],
                      numSteps=50)
dyn.run()
```

```
# initial minimization
protocol.initMinimize(dyn,
                      potList=potList,
                      numSteps=1000)
minc.run()

#from simulationTools import testGradient
#testGradient(potList,eachTerm=1)

def calcOneStructure(loopInfo):

## mod by anda: first annealing loop, to overcome high energy barriers
#    # initialize parameters for high temp dynamics.
#    InitialParams( rampedParams )
#    # high-temp dynamics setup - only need to specify parameters which
#    #   differfrom initial values in rampedParams
#    InitialParams( highTempParams )
#
#    protocol.initDynamics(dyn,
#                          initVelocities=1,
#                          bathTemp=init_t1,
#                          potList=potList,
#                          finalTime=10)
#    dyn.setETolerance( init_t1/100 ) #used to det. stepsize. default: t/1000
#    dyn.run()
#
#    # initialize parameters for cooling loop
#    InitialParams( rampedParams )
#
#    # perform simulated annealing
#    #
#    protocol.initDynamics(dyn,
#                          finalTime=0.1, #time to integrate at a given temp.
#                          numSteps=0,     # take as many steps as necessary
#                          #eTol_minimum=0.001 # cutoff for auto-TS det.
#                          )
#    anneal1.run()

# mod by anda: second annealing loop, actual annealing
    # initialize parameters for high temp dynamics.
    InitialParams( rampedParams )
    # high-temp dynamics setup - only need to specify parameters which
    #   differfrom initial values in rampedParams
    InitialParams( highTempParams )

    protocol.initDynamics(dyn,
                          initVelocities=1,
                          bathTemp=init_t2,
                          potList=potList,
                          finalTime=50)
    dyn.setETolerance( init_t2/100 ) #used to det. stepsize. default: t/1000
    dyn.run()

    # initialize parameters for cooling loop
    InitialParams( rampedParams )
```

```
    # perform simulated annealing
    #
    protocol.initDynamics(dyn,
                          finalTime=0.5,  #time to integrate at a given temp.
                          numSteps=0,     # take as many steps as necessary
                          #eTol_minimum=0.001 # cutoff for auto-TS det.
                          )
    anneal2.run()
    anneal3.run()

    #
    # torsion angle minimization
    #
    protocol.initMinimize(dyn,numSteps=5000)
    dyn.run()

    ##
    ##all atom minimization
    ##
    protocol.initMinimize(minc,potList=potList,numSteps=3000)
    minc.run()

    #
    # perform analysis and write structure
    loopInfo.writeStructure(potList,crossTerms)
    pass


from simulationTools import StructureLoop
StructureLoop(numStructures=numberOfStructures,
              startStructure=startStructure,
              structLoopAction=calcOneStructure,
              pdbTemplate=outFilename,
              genViolationStats=1,
              averageFilename="average_min.pdb",
              averageFitSel="not resname ANI and not (name H71 or name H72 or name H73)",
              averageRefineSteps=15,
              averageTopFraction=0.1,
              averagePotList=potList).run()
```

.3 Xplor-NIH calculation restraints files and structures

The coordinates of the 10 minimum-energy, violation-free structures can be found at the Protein Databank (PDB) with accession codes 2kh0 (13merHNF, face-down), 2kh1 (13merHNF, face-up), 2kz1 (13merRef) and 2kz2 (13mer2AP). The input files to the structure calculations have been deposited along with the structures.

.4 Lua scripts written for data export from CARA

The following scripts were written for use in CARA only. Their description is given in the header.

```
   language
-- script to filter through peaklist with all graded peaks and compare it
-- to normal peaklist. All peaks that are not integrated are written to
-- a new peaklist for inspection.

-- written by Andre Dallmann April-23-2007

------------------------------------------------------------------------------
------------------------------------------------------------------------------
--                                                                  Script
                                                                        --
------------------------------------------------------------------------------
------------------------------------------------------------------------------


------------------------------- PREPARATIONS ---------------------------------

-- initialize tables for peak information
t={}
t.label = {}
t.id = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}
t.atomtype = {}
t.assxlabel = {}
t.assylabel = {}


-- get Project
local ProjectNames = {}
i = 0
```

```
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName = dlg.getSymbol("Select Project","", unpack( ProjectNames ))
t.project = cara:getProject( t.ProjectName )

-- get Peaklist with all graded peaks
local PeaklistNames = {}
i = 0
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        PeaklistNames[ i ] = b:getName()
end
t.PeaklistName = dlg.getSymbol("Select Graded Peaklist","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklist = t.project:getPeakList( b:getId(a) )
        end
end

-- get Peaklist as Reference
local PeaklistNames = {}
i = 0
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        PeaklistNames[ i ] = b:getName()
end
t.PeaklistName = dlg.getSymbol("Select Reference Peaklist","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklistref = t.project:getPeakList( b:getId(a) )
        end
end

-- Get Output Filename
t.Filename = dlg.getText("Enter the output filename", "", t.ProjectName)

-- open outfile
outfile = io.output( t.Filename.."_diff.peaks" )

-- write header
outfile:write ("#Number of dimensions "..t.peaklist:getDimCount().."\n")
print ("#Number of dimensions "..t.peaklist:getDimCount())
for i = 1, t.peaklist:getDimCount() do
        outfile:write ("#INAME "..i.." "..t.peaklist:getAtomType(i).."\n")
        print ("#INAME "..i.." "..t.peaklist:getAtomType(i))
end

count = 0

------------------------------------------------------------------------------
-------------------------------- Main Body -----------------------------------
------------------------------------------------------------------------------
```

```
-- read out peak information
for i,j in pairs (t.peaklistref:getPeaks()) do --cycle through all peaks of refpeaklist
        boolean = 0
        for x,y in pairs (t.peaklist:getPeaks()) do --cycle through all peaks of graded
             peaklist
                if (j:getAssig() == y:getAssig()) then
                        boolean = 1
                end
        end
        if (boolean == 0) then
                t.peak = t.peaklistref:getPeak(i)
                count = count + 1
                t.id[i] = string.format ("%9.0f", t.peak:getId())
                t.ass = {t.peak:getAssig()}
                t.assx[i] = string.format ("%9.0f", t.ass[1])
                t.assy[i] = string.format ("%9.0f", t.ass[2])
                t.label[i] = string.format ("%25.25s", t.peak:getLabel())
                t.pos = {t.peak:getPos()}
                t.posx[i] = string.format ("%9.3f", t.pos[1])
                t.posy[i] = string.format ("%9.3f", t.pos[2])
                t.ampl[i] = string.format ("%7.0f", t.peak:getAmp())
                t.vol[i] = string.format ("%15.3f", t.peak:getVol())
                outfile:write (count..t.posx[i]..t.posy[i].." 0 U
                     "..t.vol[i]..t.ampl[i].." -    0"..t.assx[i]..t.assy[i].." 0\n#
                     "..t.label[i].."\n")
                print (count..t.posx[i]..t.posy[i].." 0 U        "..t.vol[i]..t.ampl[i].." -
                     0"..t.assx[i]..t.assy[i].." 0\n# "..t.label[i])
        end
end -- of first for loop

------------------------- End of Main Body ------------------------------------
-------------------------------------------------------------------------------

i = 0
t = nil

-- close outfiles
outfile:close()

-------------------------------------------------------------------------------
------------------------- End of Script  --------------------------------------
-------------------------------------------------------------------------------

print ( "\ncheck_intpeaks_byanda is done." )
print ( "Have a nice day!" )



-- script to export cara peaklist to Sparky

-- written by Andre Dallmann April-23-2007


-------------------------------------------------------------------------------
-------------------------------------------------------------------------------
```

```
--                                  Script                                    --
--------------------------------------------------------------------------------
--------------------------------------------------------------------------------


-------------------------------- PREPARATIONS ----------------------------------

-- initialize tables for peak information
t={}
t.label = {}
t.id = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}
t.atomtype = {}
t.assxlabel = {}
t.assylabel = {}


-- get Project
local ProjectNames = {}
i = 0
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName = dlg.getSymbol("Select Project","", unpack( ProjectNames ))
t.project = cara:getProject( t.ProjectName )

-- get Peaklist with all graded peaks
local PeaklistNames = {}
i = 0
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        PeaklistNames[ i ] = b:getName()
end
t.PeaklistName = dlg.getSymbol("Select Peaklist","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklist = t.project:getPeakList( b:getId(a) )
        end
end


-- Get Output Filename
t.Filename = dlg.getText("Enter the output filename", "", t.ProjectName)

-- open outfile
outfile = io.output( t.Filename.."_sparky.peaks" )

-- write header
```

Script code

```
outfile:write (string.format ("%25.25s","Assignment")..string.format
    ("%9.9s","w1")..string.format ("%9.9s","w1").."\n\n")
print (string.format ("%25.25s","Assignment")..string.format ("%9.9s","w1")..string.format
    ("%9.9s","w1").."\n")

count = 0

-------------------------------------------------------------------------------
------------------------------ Main Body --------------------------------------
-------------------------------------------------------------------------------

-- read out peak information
for i,peak in pairs(t.peaklist:getPeaks()) do --cycle through all peaks
                t.peak = t.peaklist:getPeak(i)
                t.label[i] = string.format ("%25.25s", t.peak:getLabel())
                t.ass = {t.peak:getAssig()}
                t.assx[i] = string.format ("%9.0f", t.ass[1])
                t.assy[i] = string.format ("%9.0f", t.ass[2])
                t.pos = {t.peak:getPos()}
                t.posx[i] = string.format ("%9.3f", t.pos[1])
                t.posy[i] = string.format ("%9.3f", t.pos[2])
                t.ampl[i] = string.format ("%7.0f", t.peak:getAmp())
                t.vol[i] = string.format ("%15.3f", t.peak:getVol())
                assx = t.project:getSpin(t.assx[i]):getLabel()
                assy = t.project:getSpin(t.assy[i]):getLabel()
                resxid = t.project:getSpin(t.assx[i]):getSystem():getId()
                resyid = t.project:getSpin(t.assy[i]):getSystem():getId()
                resx =
                    t.project:getSpin(t.assx[i]):getSystem():getResidue():getType():getShort()
                resy =
                    t.project:getSpin(t.assy[i]):getSystem():getResidue():getType():getShort()
                if (assx==assy) then
                        outfile:write(resx..resxid..assx.."-"..assy..t.posx[i]..t.posy[i].."\n")
                        print (resx..resxid..assx.."-"..assy..t.posx[i]..t.posy[i])
                else
                        outfile:write(resx..resxid..assx.."-"..resy..resyid..assy..t.posx[i]..t.posy[i].."\n"
                        print
                            (resx..resxid..assx.."-"..resy..resyid..assy..t.posx[i]..t.posy[i])
                end
        end -- of second for loop

-------------------------- End of Main Body -------------------------------------
-------------------------------------------------------------------------------

i = 0
t = nil

-- close outfiles
outfile:close()

-------------------------------------------------------------------------------
------------------------------ End of Script ----------------------------------
-------------------------------------------------------------------------------

print ( "\ntestscript_byanda is done." )
print ( "Have a nice day!" )
```

```
-- script to filter through peaklist which where exported from homoscope to
-- monoscope
-- produces two files, containing d2o and h2o peaks respectively

-- written by Andre Dallmann April-11-2007

-------------------------------------------------------------------------------
-------------------------------------------------------------------------------
--                                                                    Script
                                                                        --
-------------------------------------------------------------------------------
-------------------------------------------------------------------------------


-------------------------------- PREPARATIONS ---------------------------------

-- initialize tables for peak information
t={}
t.label = {}
t.id = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}
t.atomtype = {}
t.assxlabel = {}
t.assylabel = {}

-- Get Project
local ProjectNames = {}
i = 0
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName = dlg.getSymbol("Select Project","", unpack( ProjectNames ))
t.project = cara:getProject( t.ProjectName )

-- Get Peaklist
local PeaklistNames = {}
i = 0
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        PeaklistNames[ i ] = b:getName()
end
t.PeaklistName = dlg.getSymbol("Select Peaklist","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklist = t.project:getPeakList( b:getId(a) )
```

```
        end
end

-- Get Output Filename
t.Filename = dlg.getText("Enter the output filename", "", t.ProjectName)

-- open outfile
outfile_h2o = io.output( t.Filename.."_h2o.peaks" )
outfile_d2o = io.output( t.Filename.."_d2o.peaks" )

------------------------------------------------------------------------------
------------------------------ Main Body -------------------------------------
------------------------------------------------------------------------------

-- read out peak information
for i,peak in pairs (t.peaklist:getPeaks()) do --cycle through all peaks
        t.peak = t.peaklist:getPeak(i)
        t.id[i] = string.format ("%9.0f", t.peak:getId())
        t.ass = {t.peak:getAssig()}
        t.assx[i] = string.format ("%9.0f", t.ass[1])
        t.assy[i] = string.format ("%9.0f", t.ass[2])
        t.label[i] = string.format ("%25.25s", t.peak:getLabel())
        t.pos = {t.peak:getPos()}
        t.posx[i] = string.format ("%9.3f", t.pos[1])
        t.posy[i] = string.format ("%9.3f", t.pos[2])
        t.ampl[i] = string.format ("%7.0f", t.peak:getAmp())
        t.vol[i] = string.format ("%15.3f", t.peak:getVol())
end -- of first for loop

-- write header
outfile_h2o:write ("#Number of dimensions "..t.peaklist:getDimCount().."\n")
outfile_d2o:write ("#Number of dimensions "..t.peaklist:getDimCount().."\n")
--print ("#Number of dimensions "..t.peaklist:getDimCount())
for i = 1, t.peaklist:getDimCount() do
        outfile_h2o:write ("#INAME "..i.." "..t.peaklist:getAtomType(i).."\n")
        outfile_d2o:write ("#INAME "..i.." "..t.peaklist:getAtomType(i).."\n")
        --print ("#INAME "..i.." "..t.peaklist:getAtomType(i))
end

-- initialize counter variables
c_h2o = 0
c_d2o = 0

-- write peaklists
for x,assx in pairs (t.assx) do
        t.assxlabel[x] = t.project:getSpin(t.assx[x]):getLabel() -- get Peaklabel
        t.assylabel[x] = t.project:getSpin(t.assy[x]):getLabel() -- get Peaklabel
        if ((((t.assxlabel[x]=="H41") or (t.assxlabel[x]=="H42") or (t.assxlabel[x]=="H1") or
            (t.assxlabel[x]=="H3")) or ((t.assylabel[x]=="H41") or (t.assylabel[x]=="H42") or
            (t.assylabel[x]=="H1") or (t.assylabel[x]=="H3"))) and (t.assx[x]~=t.assy[x]))
            then
                c_h2o = c_h2o + 1
                outfile_h2o:write (c_h2o..t.posx[x]..t.posy[x].." 0 U
                    "..t.vol[x]..t.ampl[x].." -    0"..t.assx[x]..t.assy[x].." 0\n#
                    "..t.label[x].."\n")
                --print (c_h2o..t.posx[x]..t.posy[x].." 0 U      "..t.vol[x]..t.ampl[x].." -
```

```
                0"..t.assx[x]..t.assy[x].." 0\n# "..t.label[x].."   h2o")
        else
                if (((t.assxlabel[x]=="H2") and  ((t.assylabel[x]=="H2'") or
                    (t.assylabel[x]=="H2''") or (t.assylabel[x]=="H3'") or
                    (t.assylabel[x]=="H4'") or (t.assylabel[x]=="H5'") or
                    (t.assylabel[x]=="H5''") or (t.assylabel[x]=="H8"))) or
                    ((t.assylabel[x]=="H2") and  ((t.assxlabel[x]=="H2'") or
                    (t.assxlabel[x]=="H2''") or (t.assxlabel[x]=="H3'") or
                    (t.assxlabel[x]=="H4'") or (t.assxlabel[x]=="H5'") or
                    (t.assxlabel[x]=="H5''") or (t.assxlabel[x]=="H8")))) then
                -- leaves H6 connections because of 2AP
                        i = i + 1 -- dummy statement
                else
                        if (t.assx[x]~=t.assy[x]) then
                                c_d2o = c_d2o + 1
                                outfile_d2o:write (c_d2o..t.posx[x]..t.posy[x].." 0 U
                                    "..t.vol[x]..t.ampl[x].." -   0"..t.assx[x]..t.assy[x].."
                                    0\n# "..t.label[x].."\n")
                                --print (c_d2o..t.posx[x]..t.posy[x].." 0 U
                                    "..t.vol[x]..t.ampl[x].." -   0"..t.assx[x]..t.assy[x].."
                                    0\n# "..t.label[x].."   d2o")
                        end
                end

        end
end

------------------------------ End of Main Body ------------------------------------
------------------------------------------------------------------------------------

-- close outfiles
outfile_h2o:close()
outfile_d2o:close()

t = nil

------------------------------------------------------------------------------------
------------------------------- End of Script  -------------------------------------
------------------------------------------------------------------------------------

print ( "\nfilterpeaks_byanda is done." )
print ( "Have a nice day!" )


------------------------------ End filterpeaksbyanda
-------------------------------



-- script to filter through peaklist which where exported from homoscope to
-- monoscope
-- produces several peaklists, containing d2o and h2o peaks respectively
-- adjustments need to be made for unnatural nucleobases, peakwidths, and home spectrum for
     h2o and d2o!!!

-- written by Andre Dallmann May-11-2007
```

```
-------------------------------------------------------------------------------
-------------------------------------------------------------------------------
--                                  Script                                   --
-------------------------------------------------------------------------------
-------------------------------------------------------------------------------


------------------------------- PREPARATIONS ----------------------------------

-- initialize tables for peak information
t={}
t.label = {}
t.id = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}
t.atomtype = {}
t.assxlabel = {}
t.assylabel = {}

-- Get Project
local ProjectNames = {}
i = 0
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName = dlg.getSymbol("Select Project","", unpack( ProjectNames ))
t.project = cara:getProject( t.ProjectName )

-- Get Peaklist
local PeaklistNames = {}
i = 0
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        PeaklistNames[ i ] = b:getName()
end
t.PeaklistName = dlg.getSymbol("Select Peaklist H2O","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklist_h2o = t.project:getPeakList( b:getId(a) )
        end
end
i=0
t.PeaklistName = dlg.getSymbol("Select Peaklist D2O","", unpack( PeaklistNames ))
for a,b in pairs(t.project:getPeakLists()) do
        i = i + 1
        if (b:getName()==t.PeaklistName) then
                t.peaklist_d2o = t.project:getPeakList( b:getId(a) )
        end
```

```
end


-------------------------------------------------------------------------------
------------------------------- Main Body -------------------------------------
-------------------------------------------------------------------------------

------------------------------- h2o peaks -------------------------------------


-- initialize variables
local c = 0
local d = 0
local e = 0
local f = 0
local waminox = 0.027 -- peak width for amino
local wrestx = 0.027 -- peak width for rest in x dim
local wally = 0.027 -- peak width for y dim
local h2o_spectrum = 6 -- home spectrum needs to be adjusted!!!


for i,p in pairs (t.peaklist_h2o:getPeaks()) do --cycle through all peaks
        t.posx_ref,t.posy_ref = p:getPos() -- get Positions of Reference peak
        t.assx,t.assy = p:getAssig() -- get Assignment of Reference peak
        t.x = t.project:getSpin(t.assx):getLabel() -- get Peaklabel
        t.y = t.project:getSpin(t.assy):getLabel() -- get Peaklabel
        t.HNFx = t.project:getSpin(t.assx):getSystem():getResidue():getId() -- get
            Residuenumber
        t.HNFy = t.project:getSpin(t.assy):getSystem():getResidue():getId() -- get
            Residuenumber
        if (((t.x=="H41") and (t.y=="H42")) or ((t.x=="H42") and (t.y=="H41"))) and not
            ((t.HNFx==2) or (t.HNFx==12) or (t.HNFy==2) or (t.HNFy==12) or (t.HNFx==14) or
            (t.HNFx==26) or (t.HNFy==14) or (t.HNFy==26)) then
           -- selects all H41/H42 pairs which are not subjet to base pair fraying
                e = e + 1
                if (e==1) then -- for first match establish peaklist
                        peaklist3 = spec.createPeakList("1H","1H")
                        peaklist3:setName(t.project:getName().."_h41h42_h2o")
                        peaklist3:setHome(t.project:getSpectrum(h2o_spectrum))
                        t.project:addPeakList(peaklist3)
                        peaklist3:getModel(0):setWidth(1,waminox)
                        peaklist3:getModel(0):setWidth(2,wally)
                        peaklist3:setAttr("WidthX",waminox)
                        peaklist3:setAttr("WidthY",wally)
                end
                peak = peaklist3:createPeak(p:getPos()) -- create Peaks in Peaklist
                peak:setAssig(p:getAssig())
                peak:setLabel(p:getLabel())
                for xx,yy in pairs (t.peaklist_h2o:getPeaks()) do --cycle through all peaks
                        t.posx,t.posy = yy:getPos() -- get Positions of peak
                        t.assxx,t.assyy = yy:getAssig() -- get Assignment of peak
                        if (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                            (2*wally)) then -- if next peak is more than double peakwidth
                            away, isolated peak
                                if ((peak:getAttr("grade")~="b") and
                                    (peak:getAttr("grade")~="c")) then
```

```
                                peak:setAttr("grade","a")
                            end
                        elseif (wally <
                            ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                            (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            (2*wally)) then
                                if (peak:getAttr("grade")~="c") then
                                    peak:setAttr("grade","b")
                                end
                        elseif (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            wally) and (i~=xx) then -- very close proximity to next peak,
                            great error
                                peak:setAttr("grade","c")
                        end
                end
        -- this procedure is repeated for all peaks of interest
        -- different peaklists are required for peaks with different peakwidths
        -- for h2o peaks only one half of the diagonal is used
        elseif ((t.x=="H41")) and ((t.y=="H1") or (t.y=="H3") or (t.y=="H5") or (t.HNFy==7))
            and (t.assx~=t.assy) then
           -- selects all relevant H41-peaks
                c = c + 1
                if (c==1) then
                        peaklist = spec.createPeakList("1H","1H")
                        peaklist:setName(t.project:getName().."_H41_h2o")
                        peaklist:setHome(t.project:getSpectrum(h2o_spectrum))
                        t.project:addPeakList(peaklist)
                        peaklist:getModel(0):setWidth(1,waminox)
                        peaklist:getModel(0):setWidth(2,wally)
                        peaklist:setAttr("WidthX",waminox)
                        peaklist:setAttr("WidthY",wally)
                end
                peak = peaklist:createPeak(p:getPos())
                peak:setAssig(p:getAssig())
                peak:setLabel(p:getLabel())
                for xx,yy in pairs (t.peaklist_h2o:getPeaks()) do --cycle through all peaks
                        t.posx,t.posy = yy:getPos()
                        t.assxx,t.assyy = yy:getAssig()
                        if ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                            (2*wally)) then -- if peak next peak is more than double
                            peakwidth away, isolated peak
                                if ((peak:getAttr("grade")~="b") and
                                    (peak:getAttr("grade")~="c")) then
                                        peak:setAttr("grade","a")
                                end
                        elseif (wally <
                            ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                            (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            (2*wally)) then
                                if (peak:getAttr("grade")~="c") then
                                        peak:setAttr("grade","b")
                                end
                        elseif (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            wally) and (i~=xx) then -- very close proximity to next peak,
                            great error
                                peak:setAttr("grade","c")
```

```
            end
          end
elseif ((t.x=="H42")) and ((t.y=="H1") or (t.y=="H3") or (t.y=="H5") or (t.HNFy==7))
    and (t.assx~=t.assy) then
  -- selects all relevant H42 peaks
    f = f + 1
    if (f==1) then
            peaklist4 = spec.createPeakList("1H","1H")
            peaklist4:setName(t.project:getName().."_H42_h2o")
            peaklist4:setHome(t.project:getSpectrum(h2o_spectrum))
            t.project:addPeakList(peaklist4)
            peaklist4:getModel(0):setWidth(1,waminox)
            peaklist4:getModel(0):setWidth(2,wally)
            peaklist4:setAttr("WidthX",waminox)
            peaklist4:setAttr("WidthY",wally)
    end
    peak = peaklist4:createPeak(p:getPos())
    peak:setAssig(p:getAssig())
    peak:setLabel(p:getLabel())
    for xx,yy in pairs (t.peaklist_h2o:getPeaks()) do --cycle through all peaks
            t.posx,t.posy = yy:getPos()
            t.assxx,t.assyy = yy:getAssig()
            if (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                (2*wally)) then -- if peak next peak is more than double
                peakwidth away, isolated peak
                    if ((peak:getAttr("grade")~="b") and
                        (peak:getAttr("grade")~="c")) then
                            peak:setAttr("grade","a")
                    end
            elseif (wally <
                ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                (2*wally)) then
                    if (peak:getAttr("grade")~="c") then
                            peak:setAttr("grade","b")
                    end
            elseif (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                wally) and (i~=xx) then -- very close proximity to next peak,
                great error
                    peak:setAttr("grade","c")
            end
    end
elseif ((t.x=="H1" or t.x=="H3" or t.x=="H2") and (t.y=="H1" or t.y=="H3" or
    t.y=="H5" or t.HNFy==7)) and (t.assx~=t.assy) then
  -- selects all other exchangable proton peaks plus HNF-crosspeaks
    d = d + 1
    if (d==1) then
            peaklist2 = spec.createPeakList("1H","1H")
            peaklist2:setName(t.project:getName().."_rest_h2o")
            peaklist2:setHome(t.project:getSpectrum(h2o_spectrum))
            t.project:addPeakList(peaklist2)
            peaklist2:getModel(0):setWidth(1,wrestx)
            peaklist2:getModel(0):setWidth(2,wally)
            peaklist2:setAttr("WidthX",wrestx)
            peaklist2:setAttr("WidthY",wally)
    end
```

```
                peak = peaklist2:createPeak(p:getPos())
                peak:setAssig(p:getAssig())
                peak:setLabel(p:getLabel())
                for xx,yy in pairs (t.peaklist_h2o:getPeaks()) do --cycle through all peaks
                        t.posx,t.posy = yy:getPos()
                        t.assxx,t.assyy = yy:getAssig()
                        if (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                            (2*wally)) then -- if peak next peak is more than double
                            peakwidth away, isolated peak
                                if ((peak:getAttr("grade")~="b") and
                                    (peak:getAttr("grade")~="c")) then
                                        peak:setAttr("grade","a")
                                end
                        elseif (wally <
                            ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                            (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            (2*wally)) then
                                if (peak:getAttr("grade")~="c") then
                                        peak:setAttr("grade","b")
                                end
                        elseif (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                            wally) and (i~=xx) then -- very close proximity to next peak,
                            great error
                                peak:setAttr("grade","c")
                        end
                end
        end
end -- of first for loop



------------------------------ d2o peaks ----------------------------------------

function find2x (index) -- function to format the atomlabels
        local Boolean = false
        local Booleanx = false
        local Booleany = false
        for x in string.gfind(t.x,"H[2345]'[']*") do
                Booleanx=true
        end
        for y in string.gfind(t.y,"H[25]$") do
                Booleany=true
        end
        if (Booleanx==true) and (Booleany==true) then
                Boolean=true
        else
                Boolean=false
        end
        return Boolean
end

function find2y (index) -- function to filter atoms
        local Boolean = false
        local Booleanx = false
        local Booleany = false
```

```
        for x in string.gfind(t.x,"H[25]$") do
                Booleanx=true
        end
        for y in string.gfind(t.y,"H[2345]'[']*") do
                Booleany=true
        end
        if (Booleanx==true) and (Booleany==true) then
                Boolean=true
        else
                Boolean=false
        end
        return Boolean
end


-- initialize variables
local c = 0
local d = 0
local e = 0
local f = 0
local wh2 = 0.040 -- peak width for H2' or H2''
local wrest = 0.025 -- peak width for rest
local d2o_spectrum = 1

for i,p in pairs (t.peaklist_d2o:getPeaks()) do --cycle through all peaks
        t.posx_ref,t.posy_ref = p:getPos()
        t.assx,t.assy = p:getAssig()
        t.x = t.project:getSpin(t.assx):getLabel() -- get Peaklabel
        t.y = t.project:getSpin(t.assy):getLabel() -- get Peaklabel
        if (t.x=="H41") or (t.x=="H42") or (t.x=="H1") or (t.x=="H3") or (t.y=="H41") or
            (t.y=="H42") or (t.y=="H1") or (t.y=="H3") then
        t.peaklist_d2o:removePeak(p) -- remove all h2o-peaks

-- selection process for different peaklists as in h2o, repeated several times

        elseif ((t.x=="H2'") or (t.x=="H2''")) and (t.assx~=t.assy) and (find2x(i)==false) and
            (find2y(i)==false) then
           -- selects all peaks including H2' to H2'' spins on x-axis (differentiation
                necessary because of different peakwidth for y and x axis)
                d = d +1
                if (d==1) then
                        peaklist2 = spec.createPeakList("1H","1H")
                        peaklist2:setName(t.project:getName().."_h2inx_d2o")
                        peaklist2:setHome(t.project:getSpectrum(d2o_spectrum))
                        t.project:addPeakList(peaklist2)
                        peaklist2:getModel(0):setWidth(1,wh2)
                        peaklist2:getModel(0):setWidth(2,wrest)
                        peaklist:setAttr("WidthX",wh2)
                        peaklist:setAttr("WidthY",wrest)
                end
                peak = peaklist2:createPeak(p:getPos())
                peak:setAssig(p:getAssig())
                peak:setLabel(p:getLabel())
                for xx,yy in pairs (t.peaklist_d2o:getPeaks()) do --cycle through all peaks
                        t.posx,t.posy = yy:getPos()
                        t.assxx,t.assyy = yy:getAssig()
```

```
                    if ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                        (2*wh2)) then -- if peak next peak is more than double peakwidth
                        away, isolated peak
                            if ((peak:getAttr("grade")~="b") and
                                (peak:getAttr("grade")~="c")) then
                                    peak:setAttr("grade","a")
                            end
                    elseif (wh2 <
                        ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                        ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                        (2*wh2)) then
                            if (peak:getAttr("grade")~="c") then
                                    peak:setAttr("grade","b")
                            end
                    elseif ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                        wh2) and (i~=xx) then -- very close proximity to next peak, great
                        error
                            peak:setAttr("grade","c")
                    end
            end
    elseif ((t.y=="H2'") or (t.y=="H2'")) and (t.assx~=t.assy) and (find2x(i)==false) and
        (find2y(i)==false) then
      -- selects all peaks including H2' to H2'' spins on y-axis (differentiation
            necessary because of different peakwidth for y and x axis)
            e = e +1
            if (e==1) then
                    peaklist3 = spec.createPeakList("1H","1H")
                    peaklist3:setName(t.project:getName().."_h2iny_d2o")
                    peaklist3:setHome(t.project:getSpectrum(d2o_spectrum))
                    t.project:addPeakList(peaklist3)
                    peaklist3:getModel(0):setWidth(1,wrest)
                    peaklist3:getModel(0):setWidth(2,wh2)
                    peaklist:setAttr("WidthX",wrest)
                    peaklist:setAttr("WidthY",wh2)
            end
            peak = peaklist3:createPeak(p:getPos())
            peak:setAssig(p:getAssig())
            peak:setLabel(p:getLabel())
            for xx,yy in pairs (t.peaklist_d2o:getPeaks()) do --cycle through all peaks
                    t.posx,t.posy = yy:getPos()
                    t.assxx,t.assyy = yy:getAssig()
                    if ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                        (2*wh2)) then -- if peak next peak is more than double peakwidth
                        away, isolated peak
                            if ((peak:getAttr("grade")~="b") and
                                (peak:getAttr("grade")~="c")) then
                                    peak:setAttr("grade","a")
                            end
                    elseif (wh2 <
                        ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                        ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                        (2*wh2)) then
                            if (peak:getAttr("grade")~="c") then
                                    peak:setAttr("grade","b")
                            end
                    elseif ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
```

```
                        wh2) and (i~=xx) then -- very close proximity to next peak, great
                        error
                            peak:setAttr("grade","c")
                    end
            end
        elseif (t.assx~=t.assy) and (find2x(i)==false) and (find2y(i)==false) then
           -- select all other peaks which are not sugar to H2'* or H5'* peaks
            f = f +1
            if (f==1) then
                    peaklist4 = spec.createPeakList("1H","1H")
                    peaklist4:setName(t.project:getName().."_rest_d2o")
                    peaklist4:setHome(t.project:getSpectrum(d2o_spectrum))
                    t.project:addPeakList(peaklist4)
                    peaklist4:getModel(0):setWidth(1,wrest)
                    peaklist4:getModel(0):setWidth(2,wrest)
                    peaklist:setAttr("WidthX",wrest)
                    peaklist:setAttr("WidthY",wrest)
            end
            peak = peaklist4:createPeak(p:getPos())
            peak:setAssig(p:getAssig())
            peak:setLabel(p:getLabel())
            for xx,yy in pairs (t.peaklist_d2o:getPeaks()) do --cycle through all peaks
                    t.posx,t.posy = yy:getPos()
                    t.assxx,t.assyy = yy:getAssig()
                    if (((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) >
                        (2*wrest)) then
                            if ((peak:getAttr("grade")~="b") and
                                (peak:getAttr("grade")~="c")) then
                                    peak:setAttr("grade","a")
                            end
                    elseif (wrest <
                        ((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2))) and
                        ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                        (2*wrest)) then
                            if (peak:getAttr("grade")~="c") then
                                    peak:setAttr("grade","b")
                            end
                    elseif ((((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)) <
                        wrest) and (i~=xx) then
                            peak:setAttr("grade","c")
                            -- print (p:getLabel().."  "..yy:getLabel()..
                                "..((((t.posx_ref-t.posx)^2)+((t.posy_ref-t.posy)^2))^(1/2)))
                    end
            end
        end
end -- of first for loop

------------------------------ End of Main Body ------------------------------------
------------------------------------------------------------------------------------

-- t.Project:removePeakList(peaklist_d2o)
-- t.Project:removePeakList(peaklist_h2o)
t = nil

------------------------------------------------------------------------------------
------------------------------- End of Script  -------------------------------------
```

```
-------------------------------------------------------------------------------

print ( "\nfilterpeaks_byanda is done." )
print ( "Have a nice day!" )


------------------------------ End filterpeaksbyanda
--------------------------------



-- script to generate ppm-file for input to GIFA

-------------------------------------------------------------------------------
-------------------------------------------------------------------------------
--                                  Script                                   --
-------------------------------------------------------------------------------
-------------------------------------------------------------------------------


---------------------------------- PREPARATIONS -------------------------------

-- initialize tables for peak information
t={}
t.label = {}
t.id = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}
t.atomtype = {}
t.assxlabel = {}
t.assylabel = {}


-- get Project
local ProjectNames = {}
local i = 0
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName = dlg.getSymbol("Select Project","", unpack( ProjectNames ))
t.project = cara:getProject( t.ProjectName )

-- get Input-Filename
--Sparkylist = dlg.getText( "Enter input filename", "", t.ProjectName"_sparky.peaks")

-- open input file
--io.open (SparkyList, r)

-- Get Output Filename
t.Filename = dlg.getText("Enter the output filename", "", t.ProjectName.."_gifa.ppm")
```

```
-- open outfile
outfile = io.output( t.Filename )

-- write header

for i, spin in pairs (t.project:getSpins()) do
        t.label[i]=spin:getLabel()
        if (t.label[i]=="H5") then
                t.label[i]="HQ5"
        elseif (t.label[i]=="H2") then
                t.label[i]="HQ2"
        end
end
for i, spin in pairs (t.project:getSpins()) do
        if (t.label[i]=="H7") then
                outfile:write("PPM "..spin:getSystem():getResidue():getType():getShort().."
                    "..spin:getSystem():getResidue():getId().." "..t.label[i].."1
                    "..spin:getShift().." 1\n")
                outfile:write("PPM "..spin:getSystem():getResidue():getType():getShort().."
                    "..spin:getSystem():getResidue():getId().." "..t.label[i].."2
                    "..spin:getShift().." 1\n")
                outfile:write("PPM "..spin:getSystem():getResidue():getType():getShort().."
                    "..spin:getSystem():getResidue():getId().." "..t.label[i].."3
                    "..spin:getShift().." 1\n")
        else
                outfile:write("PPM "..spin:getSystem():getResidue():getType():getShort().."
                    "..spin:getSystem():getResidue():getId().." "..t.label[i].."
                    "..spin:getShift().." 1\n")
        end
end

outfile:close()
t={}

print ("done with gifappm")



-- script to remove peaklists, peaklists are picked by their names

-- written by Andre Dallmann

for i,p in pairs(cara:getProject():getPeakLists()) do
        temp = string.gfind(p:getName(),"13merHNF")
        for y in temp do
                cara:getProject():removePeakList(p)
                print (y)
        end
end

print ("peaklists removed!")



-- writes 1H shifts from different spins to separate columns in an external file
```

```
-- written by Andre Dallmann

function Format( Number ) -- function to format the chemical shifts
        FormattedNumber = string.format( "%7.3f", Number )
        return FormattedNumber
end
function Format2( String ) -- function to format the chemical shifts
        FormattedString = string.format( "%7.7s", String )
        return FormattedString
end

-- User editable parameters are below: ==========================

-- Spacer between elements of table to write out:
Spacer  = " "
Spacer2 = "   "

-- Table of spin labels whose shifts should be written to a column

SpinsInColumns = {}
SpinsInColumns[ 1 ] = "H1'"
SpinsInColumns[ 2 ] = "H2'"
SpinsInColumns[ 3 ] = "H2''"
SpinsInColumns[ 4 ] = "H3'"
SpinsInColumns[ 5 ] = "H4'"
SpinsInColumns[ 6 ] = "H5'"
SpinsInColumns[ 7 ] = "H5''"
SpinsInColumns[ 8 ] = "H1"
SpinsInColumns[ 9 ] = "H2"
SpinsInColumns[ 10 ] = "H3"
SpinsInColumns[ 11 ] = "H41"
SpinsInColumns[ 12 ] = "H42"
SpinsInColumns[ 13 ] = "H5"
SpinsInColumns[ 14 ] = "H6"
SpinsInColumns[ 15 ] = "H7"
SpinsInColumns[ 16 ] = "H8"
SpinsInColumns[ 17 ] = "H4"
SpinsInColumns[ 18 ] = "H1''"

-- End of user editable section ================================
-- define a table of temporary script variables
t={}

-- Get Parameters from User

-- 1. Get Project: ---------------------------------------------
local projectnames = {}
i=0
for ProjName,Proj in pairs( cara:getProjects() ) do
        i = i + 1
        projectnames[ i ] = ProjName
end

if i==1 then
        t.ProjectName = projectnames[ i ]
```

```
else
        t.ProjectName = dlg.getSymbol( "Choose project", "select one", unpack( projectnames ) )
end
if not t.ProjectName then
        error( "No project name defined")
else
        t.P = cara:getProject( t.ProjectName )
end

-- 2. Get Output filename: ------------------------------------------
t.FileName = dlg.getText( "Enter output filename", "output filename","shiftstable.txt" )

--3. Get Label of Spin to write out chemical shifts from
-- I replaced this step with a table , see the top of the script
--t.Label = dlg.getText( "Enter label of the spins whose chemical shifts you want to write out
    : ", "Enter Label of spins whose shifts will be written out (e.g. HA): ")


-- loop through the sequence and for each residue, create a Line
-- then for each Line look for each column entry in turn
-- add it to the end of the growing line

Seq = t.P:getSequence()
j = 0
Lines = {}
for ResId,Res in pairs( Seq ) do
        Sys = Res:getSystem()
        if Sys then -- if residue is assigned
                SpinsInSys = Sys:getSpins()
                j = j + 1
                Lines[ j ] = string.format ("%-7.7s",ResId)
                for k = 1,table.getn( SpinsInColumns ) do
                        LabelToFind = SpinsInColumns[ k ]
                        MatchingSpin = nil -- reset to none found
                        for SpinId,Spin in pairs( SpinsInSys ) do -- search for a match to
                            LabelToFind
                                if Spin:getLabel() == LabelToFind then
                                        MatchingSpin = Spin
                                end -- if Spins label matches LabelToFind
                        end -- for all Spins in System (look for match to this Label)
                        if MatchingSpin then
                                FormShift = Format( MatchingSpin:getShift() )
                                Lines[ j ] = Lines[ j ]..FormShift
                        else
                                Formzero = Format2( "-" )
                                Lines[ j ] = Lines[ j ]..Formzero -- no shift assigned, leave
                                    empty
                        end
                end -- for all elements k of SpinsInColumns (try to find a shift for this
                    label)
                --Lines[ j ] = Lines[ j ].."\n" -- next line
        end -- if System is assigned
end -- for all residues in sequence

--create string "Table" with lines
```

Script code

```
--create the first line of table
for m = 1,table.getn( SpinsInColumns ) do
        if m == 1 then
                Labels = string.format ("%7.7s", SpinsInColumns[ 1 ])
        else
                Formlabels = string.format ("%7.7s", SpinsInColumns[ m ] )
                Labels = Labels..Formlabels
        end
end
res = string.format ("%-7.7s","Res")
Header = res..Labels

for l=1,table.getn( Lines ) do
        if l==1 then
                Table = Header.."\n"..Lines[ l ]
        else
                Table = Table.."\n"..Lines[ l ]
        end
end

-- Now write out all lines to a file
file = io.open( t.FileName, "w" )
file:write( Table )
file:flush()
file:close()


print("Wrote out "..table.getn( Lines )..." lines to file "..t.FileName )
print( "script WriteShiftsInColumns is done" )
t = nil



-- writes 1H shifts from different spins to separate columns in an external file

-- written by Andre Dallmann

function Format( Number ) -- function to format the chemical shifts
        FormattedNumber = string.format( "%11.3f", Number )
        return FormattedNumber
end
function Format2( String ) -- function to format the chemical shifts
        FormattedString = string.format( "%11.11s", String )
        return FormattedString
end

-- User editable parameters are below: ==========================

-- Spacer between elements of table to write out:
Spacer  = " "
Spacer2 = "   "

-- Table of spin labels whose shifts should be written to a column

SpinsInColumns = {}
SpinsInColumns[ 1 ] = "C1'"
```

```
SpinsInColumns[ 2 ] = "C2'"
SpinsInColumns[ 3 ] = "C3'"
SpinsInColumns[ 4 ] = "C4'"
SpinsInColumns[ 5 ] = "C5'"
SpinsInColumns[ 6 ] = "C5"
SpinsInColumns[ 7 ] = "C6"
SpinsInColumns[ 8 ] = "C8"
SpinsInColumns[ 9 ] = "C1"
SpinsInColumns[ 10 ] = "C2"
SpinsInColumns[ 11 ] = "C3"
SpinsInColumns[ 12 ] = "C4"


-- End of user editable section ========================================
-- define a table of temporary script variables
t={}

-- Get Parameters from User

-- 1. Get Project: ----------------------------------------------------
local projectnames = {}
i=0
for ProjName,Proj in pairs( cara:getProjects() ) do
        i = i + 1
        projectnames[ i ] = ProjName
end

if i==1 then
        t.ProjectName = projectnames[ i ]
else
        t.ProjectName = dlg.getSymbol( "Choose project", "select one", unpack( projectnames ) )
end
if not t.ProjectName then
        error( "No project name defined")
else
        t.P = cara:getProject( t.ProjectName )
end

-- 2. Get Output filename: --------------------------------------------
t.FileName = dlg.getText( "Enter output filename", "output filename","shiftstable_C.txt" )

--3. Get Label of Spin to write out chemical shifts from
-- I replaced this step with a table , see the top of the script
--t.Label = dlg.getText( "Enter label of the spins whose chemical shifts you want to write out
    : ", "Enter Label of spins whose shifts will be written out (e.g. HA): ")


-- loop through the sequence and for each residue, create a Line
-- then for each Line look for each column entry in turn
-- add it to the end of the growing line

Seq = t.P:getSequence()
j = 0
Lines = {}
for ResId,Res in pairs( Seq ) do
        Sys = Res:getSystem()
```

```
        if Sys then -- if residue is assigned
                SpinsInSys = Sys:getSpins()
                j = j + 1
                Lines[ j ] = string.format ("%-11.11s",ResId)
                for k = 1,table.getn( SpinsInColumns ) do
                        LabelToFind = SpinsInColumns[ k ]
                        MatchingSpin = nil -- reset to none found
                        for SpinId,Spin in pairs( SpinsInSys ) do -- search for a match to
                             LabelToFind
                                if Spin:getLabel() == LabelToFind then
                                        MatchingSpin = Spin
                                end -- if Spins label matches LabelToFind
                        end -- for all Spins in System (look for match to this Label)
                        if MatchingSpin then
                                FormShift = Format( MatchingSpin:getShift() )
                                Lines[ j ] = Lines[ j ]..FormShift
                        else
                                Formzero = Format2( "-" )
                                Lines[ j ] = Lines[ j ]..Formzero -- no shift assigned, leave
                                     empty
                        end
                end -- for all elements k of SpinsInColumns (try to find a shift for this
                     label)
                --Lines[ j ] = Lines[ j ].."\n" -- next line
        end -- if System is assigned
end -- for all residues in sequence

--create string "Table" with lines

--create the first line of table
for m = 1,table.getn( SpinsInColumns ) do
        if m == 1 then
                Labels = string.format ("%11.11s", SpinsInColumns[ 1 ])
        else
                Formlabels = string.format ("%11.11s", SpinsInColumns[ m ] )
                Labels = Labels..Formlabels
        end
end
res = string.format ("%-11.11s","Res")
Header = res..Labels

for l=1,table.getn( Lines ) do
        if l==1 then
                Table = Header.."\n"..Lines[ l ]
        else
                Table = Table.."\n"..Lines[ l ]
        end
end

-- Now write out all lines to a file
file = io.open( t.FileName, "w" )
file:write( Table )
file:flush()
file:close()
```

```
print("Wrote out "..table.getn( Lines )..." lines to file "..t.FileName )
print( "script WriteShiftsInColumns is done" )
t = nil



-- First part: Script to output all chosen and integrated peaks from one project
-- and combine them in one peaklist.

-- Second part: Choose the best integrated peak among same ones or average over
-- equivalently rated peaks

-- Third part: Convert peak volumes to distances. Tricky is here the
-- differentiation of d2o and h2o and methyl peaks (all have different
-- reference peaks)!

-- Fourth part: An XPLOR-inputfile is generated where the distance information
-- and some predefined upper and lower limits (deduced from the maximum
-- deviation of the standard peaks) are used

-- written by Andre Dallmann April-05-2007, mod. May-11-2007

------------------------------------------------------------------------------
------------------------------------------------------------------------------
--                                FIRST PART                                --
------------------------------------------------------------------------------
------------------------------------------------------------------------------


------------------------------ PREPARATIONS ----------------------------------

t = {} -- table for all the variables used in the script

-- choosing one project
local ProjectNames = {}
local i = 0
for a,b in pairs(cara:getProjects()) do
        i = i + 1
        ProjectNames[ i ] = b:getName()
end
t.ProjectName=dlg.getSymbol("Select Project","", unpack( ProjectNames ) )
t.project = cara:getProject( t.ProjectName )

-- Get Output Filename
t.Filename = dlg.getText("Enter the output filename", "", t.ProjectName)

-- open outfile
outfile = io.output( t.Filename.."_all.peaks" )

-- Write header to peaklist
local label = string.format ("%25.25s", "Peaklabel")
local id = string.format ("%9.9s", "PeakID")
local assx = string.format ("%9.9s", "ID(X)")
local assy = string.format ("%9.9s", "ID(Y)")
local posx = string.format ("%9.9s", "PPM(X)")
local posy = string.format ("%9.9s", "PPM(Y)")
```

Script code

```
local ampl = string.format ("%7.7s", "Ampl")
local grade = string.format ("%9.9s", "Grade")
local vol = string.format ("%15.15s", "VolumeInt")
outfile:write("IDnew"..id..label..assx..assy..posx..posy..ampl..vol..grade.."\n")


-- generate tables for information
local count = 0
local i = 0
t.label = {}
t.id_old = {}
t.assx = {}
t.assy = {}
t.posx = {}
t.posy = {}
t.ampl = {}
t.grade = {}
t.vol = {}

-------------------------------------------------------------------------------
------------------------------- Main Body -------------------------------------
-------------------------------------------------------------------------------

-- generate list of all peaks graded abc of all peaklists in specified project
for peaklistid, peaklist in pairs(t.project:getPeakLists()) do --cycle through all peaklists
        t.peaklist = t.project:getPeakList(peaklistid)
        for peakid,peak in pairs(t.peaklist:getPeaks()) do --cycle through all peaks
                t.peak = t.peaklist:getPeak(peakid)
                if ((t.peak:getAttr("grade")== "a") or (t.peak:getAttr("grade")== "b") or
                     (t.peak:getAttr("grade")== "c")) then -- choose only peaks with grade abc
                        i = i + 1 -- this is the index for all the tables, corresponds to new
                            peakid
                        t.label[i] = string.format ("%25.25s", t.peak:getLabel())
                        t.id_old[i] = string.format ("%9.0f", t.peak:getId())
                        t.ass = {t.peak:getAssig()}
                        t.assx[i] = string.format ("%9.0f", t.ass[1])
                        t.assy[i] = string.format ("%9.0f", t.ass[2])
                        t.pos = {t.peak:getPos()}
                        t.posx[i] = string.format ("%9.3f", t.pos[1])
                        t.posy[i] = string.format ("%9.3f", t.pos[2])
                        t.ampl[i] = string.format ("%7.0f", t.peak:getAmp())
                        t.grade[i] = string.format ("%7.7s", t.peak:getAttr("grade"))
                        t.vol[i] = string.format ("%15.3f", t.peak:getVol())
                        outfile:write(i.."
                            "..t.id_old[i]..t.label[i]..t.assx[i]..t.assy[i]..t.posx[i]..t.posy[i]
..t.ampl[i]..t.vol[i]..t.grade[i].."\n")
                end --of if loop
        end -- of second for loop
end -- of first for loop

------------------------------ End of Main Body -------------------------------
-------------------------------------------------------------------------------

-- close outfile
outfile:close()
```

```
-------------------------------------------------------------------------------
---------------------------- End of FIRST PART --------------------------------
-------------------------------------------------------------------------------



-------------------------------------------------------------------------------
-------------------------------------------------------------------------------
--                              SECOND PART                                  --
-------------------------------------------------------------------------------
-------------------------------------------------------------------------------


------------------------------- PREPARATIONS ----------------------------------

-- open outfile
outfile = io.output( t.Filename.."_combo.peaks" )
outfile2 = io.output( t.Filename.."_negvol.peaks" )

-- initialize variables
local x = 0
local counter = 1
local a = string.format ("%7.7s","a")
local b = string.format ("%7.7s","b")
local c = string.format ("%7.7s","c")


-------------------------------------------------------------------------------
------------------------------- Main Body -------------------------------------
-------------------------------------------------------------------------------


------------------------- Preparing combination -------------------------------

for i,assx in pairs (t.assx) do
        for j,assy in pairs (t.assy) do
                if ((((t.assx[i]==t.assx[j]) and (t.assy[i]==t.assy[j])) or
                     ((t.assx[i]==t.assy[j]) and (t.assy[i] == t.assx[j]))) and not (j==i))
                     then
                -- select all peaks that have the same assignment (including cross-diagonal
                     peaks)
                        counter = counter + 1
                        if (t.grade[i]==t.grade[j]) then
                                t.vol[i] = string.format ("%15.3f",(t.vol[i] + t.vol[j])) --
                                     average volumes, rest stays
                                t.label[j] = nil -- set jth peak to nil
                                t.assx[j] = nil
                                t.assy[j] = nil
                                t.posx[j] = nil
                                t.posy[j] = nil
                                t.ampl[j] = nil
                                t.grade[j] = nil
                                t.vol[j] = nil
                                t.id_old[j] = nil
```

```
                        end
                        if (((a==t.grade[i]) and ((t.grade[j]==b) or (t.grade[j]==c))) or
                            ((b==t.grade[i]) and (t.grade[j]==c))) then
                                counter = 1
                                t.label[j] = nil -- set jth peak to nil
                                t.assx[j] = nil
                                t.assy[j] = nil
                                t.posx[j] = nil
                                t.posy[j] = nil
                                t.ampl[j] = nil
                                t.grade[j] = nil
                                t.vol[j] = nil
                                t.id_old[j] = nil
                        end
                        if (((a==t.grade[j]) and ((t.grade[i]==b) or (t.grade[i]==b))) or
                            ((b==t.grade[j]) and (t.grade[i]==c))) then
                                counter = 1
                                t.vol[i] = t.vol[j] --transfer volume and grade of better
                                    integrated peak (j)
                                t.grade[i] = t.grade[j]
                                t.label[j] = nil -- set jth peak to nil
                                t.assx[j] = nil
                                t.assy[j] = nil
                                t.posx[j] = nil
                                t.posy[j] = nil
                                t.ampl[j] = nil
                                t.grade[j] = nil
                                t.vol[j] = nil
                                t.id_old[j] = nil
                        end
                end -- if loop
        end -- second for loop
        if (counter > 1) then -- only valid if grades are the same and averaging is needed
                t.vol[i] = string.format ("%15.3f",(t.vol[i]/counter))
        end
        counter = 1
end -- first for loop


------------------------------------------------------------------------------
---------------------- Generating new combined peaklist ----------------------

-- initialize new tables for the combined peaklist
t.labelnew = {}
t.assxnew = {}
t.assynew = {}
t.assxlabel = {}
t.assylabel = {}
t.assxresid = {}
t.assyresid = {}
t.gradenew= {}
t.volnew = {}

for i,assx in pairs (t.assx) do -- generate new table with combined peaks
        t.volnum=tonumber(t.vol[i])
        if (t.volnum>0) then
```

```
                x = x + 1
                t.labelnew[x] = t.label[i]
                t.assxnew[x] = t.assx[i]
                t.assynew[x] = t.assy[i]
                t.assxlabel[x] = string.format ("%7.7s",
                    t.project:getSpin(t.assx[i]):getLabel()) -- get Peaklabel
                t.assylabel[x] = string.format ("%7.7s",
                    t.project:getSpin(t.assy[i]):getLabel()) -- get Peaklabel
                t.assxresid[x] = string.format ("%5.5s",
                    t.project:getSpin(t.assx[i]):getSystem():getResidue():getId()) -- get
                    residue id
                t.assyresid[x] = string.format ("%5.5s",
                    t.project:getSpin(t.assy[i]):getSystem():getResidue():getId()) -- get
                    residue id
                t.gradenew[x] = t.grade[i]
                t.volnew[x] = t.vol[i]
                outfile:write (x.."
                    "..t.labelnew[x]..t.assxlabel[x]..t.assylabel[x]..t.gradenew[x]..t.volnew[x].."\n")
        else
                outfile2:write (i.."
                    "..t.label[i]..t.assx[i]..t.assy[i]..t.grade[i]..t.vol[i].."\n")
        end
end

-- loop to correct for base rectangle sum method error
-- for peaks with grade c or b divide volume by 2 or 1.5 respectively
-- this is a very rough approximation!!!
for i,vol in pairs (t.volnew) do
        if (t.gradenew[i]==c) then
                t.volnew[i]=string.format("%15.3f",vol/2)
        elseif (t.gradenew[i]==b) then
                t.volnew[i]=string.format("%15.3f",vol/1.5)
        end
end

------------------------------ End of Main Body ------------------------------------
------------------------------------------------------------------------------------

-- close outfile
outfile:close()
outfile2:close()

local i = 0

------------------------------------------------------------------------------------
------------------------------ End of SECOND PART ----------------------------------
------------------------------------------------------------------------------------




------------------------------------------------------------------------------------
------------------------------------------------------------------------------------
--                                THIRD PART                                      --
------------------------------------------------------------------------------------
------------------------------------------------------------------------------------
```

```
------------------------------ PREPARATIONS ------------------------------------

-- open outfile
outfile = io.output( t.Filename.."_dist.peaks" )
outfile2 = io.output( t.Filename.."_notused.peaks" )

-- initialize variables
local sumcyt = 0
local summet = 0
local sumcytamino = 0
local sumcyth42h5 = 0
local sumcyth41h5 = 0
local countcyt = 0
local countmet = 0
local countcytamino = 0
local countcyth42h5 = 0
local countcyth41h5 = 0

--------------------------------------------------------------------------------
------------------------------ Main Body ---------------------------------------
--------------------------------------------------------------------------------


------------------ Setting up Reference Volumes and Distances ------------------

-- sum up reference peaks
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H[56]/H[56] [0-9]:C[0-9]+") do -- establish
            reference for d2o peaks
                countcyt = countcyt + 1
                sumcyt = sumcyt + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H[67]/H[67] [0-9]:T[0-9]+") do -- establish
            reference for methyl peaks
                countmet = countmet + 1
                summet = summet + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H4[12]/H4[12] [0-9]:C[0-9]+") do -- establish
            reference for h2o exchangeable peaks
                countcytamino = countcytamino + 1
                sumcytamino = sumcytamino + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H42/H5 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks
                countcyth42h5 = countcyth42h5 + 1
                sumcyth42h5 = sumcyth42h5 + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H5/H42 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks
                countcyth42h5 = countcyth42h5 + 1
                sumcyth42h5 = sumcyth42h5 + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H41/H5 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable with H41 peaks
                countcyth41h5 = countcyth41h5 + 1
```

```
                sumcyth41h5 = sumcyth41h5 + t.volnew[j]
        end
        for y in string.gfind (t.labelnew[j],"H5/H41 [0-9]:C[0-9]+") do -- establish reference
             for h2o exchangeable-non-exchangeable with H41 peaks
                countcyth41h5 = countcyth41h5 + 1
                sumcyth41h5 = sumcyth41h5 + t.volnew[j]
        end
end

refvolcyt = string.format ("%13.3f", sumcyt / countcyt) -- average volume of CYT H5-H6
refdistcyt = 2.48 -- distance of CYT H5-H6
refvolmet = string.format ("%13.3f", summet / countmet) -- average volume of THY H6-H7
refdistmet = 3.09 -- distance of THY H6-H7
refvolcytamino = string.format ("%13.3f", sumcytamino / countcytamino) -- average volume of
    CYT H41-H42
refdistcytamino = 1.70 -- distance of CYT H41-H42
refvolcyth42h5 = string.format ("%13.3f", sumcyth42h5 / countcyth42h5) -- average volume of
    CYT H42-H5
refdistcyth42h5 = 2.40 -- distance of CYT H42-H5
refvolcyth41h5 = string.format ("%13.3f", sumcyth41h5 / countcyth41h5) -- average volume of
    CYT H41-H5
refdistcyth41h5 = 3.62 -- distance of CYT H41-H5


------------------ Prepare standard deviations for references --------------------

-- initialize variables
local stddevsumcyt = 0
local stddevsummet = 0
local stddevsumcytamino = 0
local stddevsumcyth42h5 = 0
local stddevsumcyth41h5 = 0
local maxdev1 = 0
local maxdev2 = 0
local maxdev3 = 0
local maxdev4 = 0
local maxdev5 = 0

for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H[56]/H[56] [0-9]:C[0-9]+") do
                stddevsumcyt = stddevsumcyt + (t.volnew[j]-refvolcyt)^2 -- standard deviation
                dummy1 = math.abs(t.volnew[j]-refvolcyt) -- dummy for maximum deviation
                if (dummy1 > maxdev1)then
                        maxdev1 = dummy1
                end
        end
        for y in string.gfind (t.labelnew[j],"H[67]/H[67] [0-9]:T[0-9]+") do -- establish
             reference for methyl peaks
                stddevsummet = stddevsummet + (t.volnew[j]-refvolmet)^2 -- standard deviation
                dummy2 = math.abs(t.volnew[j]-refvolmet) -- dummy for maximum deviation
                if (dummy2 > maxdev2) then
                        maxdev2 = dummy2
                end
        end
        for y in string.gfind (t.labelnew[j],"H4[12]/H4[12] [0-9]:C[0-9]+") do -- establish
             reference for h2o exchangeable peaks
```

```
                stddevsumcytamino = stddevsumcytamino + (t.volnew[j]-refvolcytamino)^2 --
                    standard deviation
                dummy3 = math.abs(t.volnew[j]-refvolcytamino) -- dummy for maximum deviation
                if (dummy3 > maxdev3) then
                        maxdev3 = dummy3
                end
        end
        for y in string.gfind (t.labelnew[j],"H42/H5 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks (appears twice because of selection
            reasons)
                stddevsumcyth42h5 = stddevsumcyth42h5 + (t.volnew[j]-refvolcyth42h5)^2 --
                    standard deviation
                dummy4 = math.abs(t.volnew[j]-refvolcyth42h5) -- dummy for maximum deviation
                if (dummy4 > maxdev4) then
                        maxdev4 = dummy4
                end
        end
        for y in string.gfind (t.labelnew[j],"H5/H42 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks
                stddevsumcyth42h5 = stddevsumcyth42h5 + (t.volnew[j]-refvolcyth42h5)^2 --
                    standard deviation
                dummy4 = math.abs(t.volnew[j]-refvolcyth42h5) -- dummy for maximum deviation
                if (dummy4 > maxdev4) then
                        maxdev4 = dummy4
                end
        end
        for y in string.gfind (t.labelnew[j],"H41/H5 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks (appears twice because of selection
            reasons)
                stddevsumcyth41h5 = stddevsumcyth41h5 + (t.volnew[j]-refvolcyth41h5)^2 --
                    standard deviation
                dummy5 = math.abs(t.volnew[j]-refvolcyth41h5) -- dummy for maximum deviation
                if (dummy5 > maxdev5) then
                        maxdev5 = dummy5
                end
        end
        for y in string.gfind (t.labelnew[j],"H5/H41 [0-9]:C[0-9]+") do -- establish reference
            for h2o exchangeable-non-exchangeable peaks
                stddevsumcyth41h5 = stddevsumcyth41h5 + (t.volnew[j]-refvolcyth41h5)^2 --
                    standard deviation
                dummy5 = math.abs(t.volnew[j]-refvolcyth41h5) -- dummy for maximum deviation
                if (dummy5 > maxdev5) then
                        maxdev5 = dummy5
                end
        end

end

---------------- Calculate standard deviations for references -------------------

stddevcyt = string.format ("%13.3f", (stddevsumcyt / countcyt)^(1/2))
stddevmet = string.format ("%13.3f", (stddevsummet / countmet)^(1/2))
stddevcytamino = string.format ("%13.3f", (stddevsumcytamino / countcytamino)^(1/2))
stddevcyth42h5 = string.format ("%13.3f", (stddevsumcyth42h5 / countcyth42h5)^(1/2))
stddevcyth41h5 = string.format ("%13.3f", (stddevsumcyth41h5 / countcyth41h5)^(1/2))
```

```
---------- Prepare maximum deviations in percent for references ----------

if (maxdev1==nil) then
        maxdevcyt = string.format ("%13.3f", dummy1/refvolcyt)
else
        maxdevcyt = string.format ("%13.3f", maxdev1/refvolcyt)
end
if (maxdev2==nil) then
        maxdevmet = string.format ("%13.3f", dummy2/refvolmet)
else
        maxdevmet = string.format ("%13.3f", maxdev2/refvolmet)
end
if (maxdev3==nil) then
        maxdevcytamino = string.format ("%13.3f", dummy3/refvolcytamino)
else
        maxdevcytamino = string.format ("%13.3f", maxdev3/refvolcytamino)
end
if (maxdev4==nil) then
        maxdevcyth42h5 = string.format ("%13.3f", dummy4/refvolcyth42h5)
else
        maxdevcyth42h5 = string.format ("%13.3f", maxdev4/refvolcyth42h5)
end
if (maxdev5==nil) then
        maxdevcyth41h5 = string.format ("%13.3f", dummy5/refvolcyth41h5)
else
        maxdevcyth41h5 = string.format ("%13.3f", maxdev5/refvolcyth41h5)
end


---------- Prepare for distance calculation ----------

function f ( String ) -- function to format the atomlabels
        FormattedString = string.format( "%7.7s", String )
        return FormattedString
end
function f2 ( String ) -- function to format the atomlabels
        FormattedString = string.format( "%9.9s", String )
        return FormattedString
end
function d2ox ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        for x in string.gfind(t.assxlabel[index],"H[12345]'[']*") do
                Boolean=true
        end
        for y in string.gfind(t.assxlabel[index],"H[2568]") do
                Boolean=true
        end
        return Boolean
end
function d2oy ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        for x in string.gfind(t.assylabel[index],"H[12345]'[']*") do
                Boolean=true
        end
        for y in string.gfind(t.assylabel[index],"H[2568]") do
                Boolean=true
```

```
        end
        return Boolean
end
function h2ox ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        for x in string.gfind(t.assxlabel[index],"H[13]") do
                Boolean=true
        end
        return Boolean
end
function h2oy ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        for x in string.gfind(t.assylabel[index],"H[13]") do
                Boolean=true
        end
        return Boolean
end
function HNFx ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        local resid=t.project:getSpin(t.assxnew[index]):getSystem():getResidue():getId()
        if resid==7 or resid==20 then
                Boolean=true
        end
        return Boolean
end
function HNFy ( index ) -- function to find non-exchangeable protons on x axis
        local Boolean=false
        local resid=t.project:getSpin(t.assynew[index]):getSystem():getResidue():getId()
        if resid==7 or resid==20 then
                Boolean=true
        end
        return Boolean
end

-- initialize new tables for distance and the lower and upper limit (same)
t.distance = {}
t.limit = {}
local i = nil
local assx = nil

------------------------ Distance and Limit calculation --------------------------

-- select atom pairs corresponding to references and calculate distances and
-- limits and write out new peaklist to file

-- limits are calculated by taking the maximum deviation of the corresponding
--reference peak times the distance


for i,assx in pairs (t.assxlabel) do
   if (((HNFx(i)==true) or (d2ox(i)==true)) and ((HNFy(i)==true) or (d2oy(i)==true))) then
                t.distance[i] = string.format ("%7.2f",
                     refdistcyt*(refvolcyt/t.volnew[i])^(1/6))
                if (t.gradenew[i]==a) then -- error bounds scaled by grading of integration
                        t.limit[i] = string.format ("%7.1f", t.distance[i]*maxdevcyt)
                else
```

```
                if (t.gradenew[i]==b) then
                        t.limit[i] = string.format ("%7.1f",
                             t.distance[i]*maxdevcyt*1.2)
                else
                        t.limit[i] = string.format ("%7.1f",
                             t.distance[i]*maxdevcyt*1.4)
                end
            end
            outfile:write (f2("d2o:  ")..f(i)..t.labelnew[i]..assx..t.assylabel[i]
..t.gradenew[i]..t.volnew[i]..t.distance[i]..t.limit[i].."\n")
        elseif ((assx==f("H7")) or (t.assylabel[i]==f("H7"))) then
            t.distance[i] = string.format ("%7.2f",
                 refdistmet*(refvolmet/t.volnew[i])^(1/6))
            if (t.gradenew[i]==a) then -- error bounds scaled by grading of integration
                    t.limit[i] = string.format ("%7.1f", t.distance[i]*maxdevmet)
            else
                    if (t.gradenew[i]==b) then
                            t.limit[i] = string.format ("%7.1f",
                                 t.distance[i]*maxdevmet*1.2)
                    else
                            t.limit[i] = string.format ("%7.1f",
                                 t.distance[i]*maxdevmet*1.4)
                    end
            end
            outfile:write (f2("methyl:  ")..f(i)..t.labelnew[i]..assx
..t.assylabel[i]..t.gradenew[i]..t.volnew[i]..t.distance[i]..t.limit[i].."\n")
        elseif (((HNFx(i)==true) or (h2ox(i)==true) or (d2ox(i)==true)) and ((h2oy(i)==true)
             or (t.assylabel[i]==f("H42")))) or (((h2ox(i)==true) or (assx==f("H42"))) and
             ((HNFy(i)==true) or (h2oy(i)==true) or (d2oy(i)==true))) then
            t.distance[i] = string.format ("%7.2f",
                 refdistcyth42h5*(refvolcyth42h5/t.volnew[i])^(1/6))
            if (t.gradenew[i]==a) then -- error bounds scaled by grading of integration
                    t.limit[i] = string.format ("%7.1f", t.distance[i]*maxdevcyth42h5)
                    else
                    if (t.gradenew[i]==b) then
                            t.limit[i] = string.format ("%7.1f",
                                 t.distance[i]*maxdevcyth42h5*1.2)
                    else
                            t.limit[i] = string.format ("%7.1f",
                                 t.distance[i]*maxdevcyth42h5*1.4)
                    end
            end
            outfile:write (f2("d2o_h2o:  ")..f(i)..t.labelnew[i]..assx..
t.assylabel[i]..t.gradenew[i]..t.volnew[i]..t.distance[i]..t.limit[i].."\n")

        elseif (((HNFx(i)==true) or (h2ox(i)==true) or (d2ox(i)==true)) and
             (t.assylabel[i]==f("H41"))) or ((assx==f("H41")) and ((HNFy(i)==true) or
             (h2oy(i)==true) or (d2oy(i)==true))) then
                    t.distance[i] = string.format ("%7.2f",
                         refdistcyth41h5*(refvolcyth41h5/t.volnew[i])^(1/6))
                    if (t.gradenew[i]==a) then -- error bounds scaled by grading of
                         integration
                            t.limit[i] = string.format ("%7.1f",
                                 t.distance[i]*maxdevcyth41h5)
                    else
                            if (t.gradenew[i]==b) then
```

```
                                        t.limit[i] = string.format ("%7.1f",
                                            t.distance[i]*maxdevcyth41h5*1.2)
                                    else
                                        t.limit[i] = string.format ("%7.1f",
                                            t.distance[i]*maxdevcyth41h5*1.4)
                                    end
                                end
                                outfile:write (f2("H41:
                                    ")..f(i)..t.labelnew[i]..assx..t.assylabel[i]..t.gradenew[i]
..t.volnew[i]..t.distance[i]..t.limit[i].."\n")
        else -- write out peaks not used to file!!!
                t.distance[i] = string.format ("%7.2f",
                    refdistcyt*(refvolcyt/t.volnew[i])^(1/6))
                if (t.gradenew[i]==a) then -- error bounds scaled by grading of integration
                        t.limit[i] = string.format ("%7.1f", t.distance[i]*maxdevcyt)
                else
                        if (t.gradenew[i]==b) then
                                t.limit[i] = string.format ("%7.1f",
                                    t.distance[i]*maxdevcyt*1.2)
                        else
                                t.limit[i] = string.format ("%7.1f",
                                    t.distance[i]*maxdevcyt*1.4)
                        end
                end
                outfile2:write (f2("not used (ref on C H5-H6):
                    ")..f(i)..t.labelnew[i]..assx..t.assylabel[i]..
t.gradenew[i]..t.volnew[i]..t.distance[i]..t.limit[i].."\n")
        end
end

------------------------------ End of Main Body ------------------------------
------------------------------------------------------------------------------

-- close outfile
outfile:close()
outfile2:close()

local i = 0

------------------------------------------------------------------------------
------------------------------ End of THIRD PART -----------------------------
------------------------------------------------------------------------------




------------------------------------------------------------------------------
------------------------------------------------------------------------------
--                                FOURTH PART                                --
------------------------------------------------------------------------------
------------------------------------------------------------------------------


------------------------------- PREPARATIONS ---------------------------------

-- open outfile
outfile = io.output( t.Filename.."_reference.peaks" )
```

```
function f ( String ) -- function to format the atomlabels
        FormattedString = string.format( "%7.2f", String )
        return FormattedString
end


-------------------------------------------------------------------------------
---------------------------------- Main Body ----------------------------------
-------------------------------------------------------------------------------


outfile:write
     ("\n-------------------------------------------------------------------------------------------\n\nReference
     for non-exchangeable proton cross-peaks: CYT H5-H6\n\nreference_vol  ref_dist
     standard_dev
     maximum_dev(%)\n"..refvolcyt..f(refdistcyt)..stddevcyt..maxdevcyt.."\n\n
     Peaklabel     Volume      Dist    Dev\n")
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H[56]/H[56] [0-9]:C[0-9]+") do -- reference for
             d2o peaks
                outfile:write
                     (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcyt).."\n")
        end
end
outfile:write
     ("\n-------------------------------------------------------------------------------------------\n\nReference
     for methyl proton cross-peaks: MET H6-H7\n\nreference_vol  ref_dist  standard_dev
     maximum_dev(%)\n"..refvolmet..f(refdistmet)..stddevmet..maxdevmet.."\n\n         Peaklabel
        Volume      Dist    Dev\n")
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H[67]/H[67] [0-9]:T[0-9]+") do -- reference for
             methyl peaks
                outfile:write
                     (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistmet).."\n")
        end
end
outfile:write
     ("\n-------------------------------------------------------------------------------------------\n\nReference
     for exchangeable proton cross-peaks: CYT H41-H42\n\nreference_vol  ref_dist  standard_dev

     maximum_dev(%)\n"..refvolcytamino..f(refdistcytamino)..stddevcytamino..maxdevcytamino.."\n\n
           Peaklabel     Volume      Dist    Dev\n")
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H4[12]/H4[12] [0-9]:C[0-9]+") do -- reference
             for h2o exchangeable peaks
                outfile:write
                     (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcytamino).."\n")
        end
end
outfile:write
     ("\n-------------------------------------------------------------------------------------------\n\nReference
     for non-exchangeable/exchangeable proton cross-peaks: CYT H42-H5\n\nreference_vol
     ref_dist  standard_dev
     maximum_dev(%)\n"..refvolcyth42h5..f(refdistcyth42h5)..stddevcyth42h5..maxdevcyth42h5.."\n\n
           Peaklabel     Volume      Dist    Dev\n")
```

```
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H42/H5 [0-9]:C[0-9]+") do -- reference for h2o
            exchangeable-non-exchangeable peaks (appears twice because of selection reasons)
                outfile:write
                    (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcyth42h5)..\"\n")
        end
end
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H5/H42 [0-9]:C[0-9]+") do -- reference for h2o
            exchangeable-non-exchangeable peaks
                outfile:write
                    (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcyth42h5)..\"\n")
        end
end
outfile:write
    ("\n-------------------------------------------------------------------------------------------------\n\nReference
    for H41 proton cross-peaks: CYT H41-H5\n\nreference_vol  ref_dist  standard_dev
    maximum_dev(%)\n"..refvolcyth41h5..f(refdistcyth41h5)..stddevcyth41h5..maxdevcyth41h5.."\n\n
          Peaklabel    Volume      Dist    Dev\n")
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H5/H41 [0-9]:C[0-9]+") do -- reference for
            H41-containing peaks
                outfile:write
                    (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcyth41h5)..\"\n")
        end
end
for j,assx in pairs (t.assxnew) do
        for y in string.gfind (t.labelnew[j],"H41/H5 [0-9]:C[0-9]+") do -- reference for
            H41-containing peaks
                outfile:write
                    (t.labelnew[j]..t.volnew[j]..t.distance[j]..f(t.distance[j]-refdistcyth41h5)..\"\n")
        end
end

------------------------------ End of Main Body ------------------------------
------------------------------------------------------------------------------

-- close outfile
outfile:close()

i = 0

------------------------------------------------------------------------------
------------------------------ End of FOURTH PART ----------------------------
------------------------------------------------------------------------------




------------------------------------------------------------------------------
------------------------------------------------------------------------------
--                                FIFTH PART                                --
------------------------------------------------------------------------------
------------------------------------------------------------------------------


------------------------------ PREPARATIONS ----------------------------------
```

```
-- open outfile
outfile   = io.output( t.Filename.."_xplor.list" )
outfile2  = io.output( t.Filename.."_xplor_all.list" )
outfile3  = io.output( "picktbl_"..t.Filename)
outfile4  = io.output( t.Filename.."_xplor.noe" )
outfile5  = io.output( t.Filename.."_xplor_all.noe" )
outfile6  = io.output( "picktbl_all_"..t.Filename)
outfile7  = io.output( t.Filename.."_xplor_longdist.list" )
outfile8  = io.output( t.Filename.."_xplor_longdist.noe" )
outfile9  = io.output( t.Filename.."_xplor_H1H5sug.list" )
outfile10 = io.output( t.Filename.."_xplor_H1H5sug.noe" )
outfile11 = io.output( "picktbl_longdist_"..t.Filename)
outfile12 = io.output( "picktbl_H1H5sug_"..t.Filename)

function find (index) -- function to find atomlabels
        local Boolean = false
        local Booleanx = false
        local Booleany = false
        for x in string.gfind(t.assxlabel[index],"H[2345]'[']*") do
                Booleanx=true
        end
        for y in string.gfind(t.assylabel[index],"H[2345]'[']*") do
                Booleany=true
        end
        if (Booleanx==true) and (Booleany==true) then
                Boolean=true
        else
                Boolean=false
        end
        return Boolean
end
function find_h1 (index) -- function to format the atomlabels
        local Boolean = false
        local Booleanx = false
        local Booleany = false
        for x in string.gfind(t.assxlabel[index],"H[12345]'[']*") do
                Booleanx=true
        end
        for y in string.gfind(t.assylabel[index],"H[12345]'[']*") do
                Booleany=true
        end
        if (Booleanx==true) and (Booleany==true) then
                Boolean=true
        else
                Boolean=false
        end
        return Boolean
end
function find_h1h5sug (index) -- function to format the atomlabels
        local Boolean = false
        local Booleanx = false
        local Booleany = false
        for x in string.gfind(t.assxlabel[index],"H[15]'[']*") do
                Booleanx=true
        end
```

```
        for y in string.gfind(t.assylabel[index],"H[15]'[']*") do
                Booleany=true
        end
        if (Booleanx==true) and (Booleany==true) then
                Boolean=true
        else
                Boolean=false
        end
        return Boolean
end

for i,assx in pairs (t.assxlabel) do -- iterate over all peaks
        if (t.assxlabel[i]=="H5") then
                t.assxlabel[i]="HQ5"
        elseif (t.assxlabel[i]=="H2") then
                t.assxlabel[i]="HQ2"
        elseif (t.assxlabel[i]=="H7") then
                t.assxlabel[i]="H7#"
        end
end
for i,assy in pairs (t.assylabel) do -- iterate over all peaks
        if (t.assylabel[i]=="H5") then
                t.assylabel[i]="HQ5"
        elseif (t.assylabel[i]=="H2") then
                t.assylabel[i]="HQ2"
        elseif (t.assylabel[i]=="H7") then
                t.assylabel[i]="H7#"
        end
end
for i,assx in pairs (t.assxnew) do -- iterate over all peaks
        if (t.distance[i]) and  (find(i)==false) then -- filter out negative volume peaks
                if (find_h1(i)==false) and ((t.distance[i]/1) < 4.5) then
                        outfile:write ("assign (resid"..t.assxresid[i].." and
                            name"..t.assxlabel[i].."") (resid"..t.assyresid[i].." and
                            name"..t.assylabel[i].."")"..t.distance[i]..t.limit[i]..t.limit[i].."\n")
                        outfile2:write ("assign (resid"..t.assxresid[i].." and
                            name"..t.assxlabel[i].."") (resid"..t.assyresid[i].." and
                            name"..t.assylabel[i].."")"..t.distance[i]..t.limit[i]..t.limit[i].."\n")
                        outfile3:write ("pick bond (resid"..t.assxresid[i].." and
                            name"..t.assxlabel[i].."") (resid"..t.assyresid[i].." and
                            name"..t.assylabel[i].."")".." geometry\ndisplay \$result".."\n")
                        outfile4:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]
..t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                        outfile5:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]..
t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                elseif (find_h1(i)==false) then
                        outfile2:write ("assign (resid"..t.assxresid[i].." and
                            name"..t.assxlabel[i].."") (resid"..t.assyresid[i].." and
                            name"..t.assylabel[i].."")"..t.distance[i]..t.limit[i]..t.limit[i]..
                            !added!\n")
                        outfile5:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]..
t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                        outfile6:write ("pick bond (resid"..t.assxresid[i].." and
                            name"..t.assxlabel[i].."") (resid"..t.assyresid[i].." and
                            name"..t.assylabel[i].."")".." geometry\ndisplay \$result".."\n")
                        outfile7:write ("assign (resid"..t.assxresid[i].." and
```

```
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")"..t.distance[i]..t.limit[i]..t.limit[i].."
                        !added!\n")
                    outfile8:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]
..t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                    outfile11:write ("pick bond (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")".." geometry\ndisplay \$result".."\n")
                elseif (find_h1h5sug(i)==true) then
                    outfile2:write ("assign (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")"..t.distance[i]..t.limit[i]..t.limit[i].."
                        !added!\n")
                    outfile5:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]..
t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                    outfile6:write ("pick bond (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")".." geometry\ndisplay \$result".."\n")
                    outfile9:write ("assign (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")"..t.distance[i]..t.limit[i]..t.limit[i].."
                        !added!\n")
                    outfile10:write (t.assxresid[i].. t.assxlabel[i]..t.assyresid[i]..
t.assylabel[i]..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                    outfile12:write ("pick bond (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")".." geometry\ndisplay \$result".."\n")
                else
                    outfile2:write ("assign (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")"..t.distance[i]..t.limit[i]..t.limit[i].."
                        !added!\n")
                    outfile5:write (t.assxresid[i]..
                        t.assxlabel[i]..t.assyresid[i]..t.assylabel[i]
..t.labelnew[i]..t.gradenew[i]..t.distance[i].."\n")
                    outfile6:write ("pick bond (resid"..t.assxresid[i].." and
                        name"..t.assxlabel[i]..") (resid"..t.assyresid[i].." and
                        name"..t.assylabel[i]..")".." geometry\ndisplay \$result".."\n")
                end
        end
end

------------------------------ End of Main Body ------------------------------------
------------------------------------------------------------------------------------


-- close outfile
outfile:close()
outfile2:close()
outfile3:close()
outfile4:close()
outfile5:close()
outfile6:close()
outfile7:close()
outfile8:close()
outfile9:close()
outfile10:close()
```

```
outfile11:close()
outfile12:close()

i = 0
t = nil

-------------------------------------------------------------------------------
---------------------------- End of FIFTH PART --------------------------------
-------------------------------------------------------------------------------


print ( "\ngenerateinput_byanda is done." )
print ( "Have a nice day!" )


---------------------------- End generateinput_byanda -------------------------
```

.5 Utility scripts

In this section a short collection of the most frequently used utility scripts is presented. They are grouped according to their function and a small description is supplied.

Scripts for converting program inputs/outputs for further use in other programs

The next two scripts are used to convert a SPARKY resonance table to a CARA atomlist.

```
   language
#!/bin/csh
#
# Skript um aus Sparky-resonance-table eine CARA-atomlist zu machen
#
awk 'NR==1 {print "#Number of dimensions 2"; print "INAME 1 H"; print
  "INAME 2 H"} {print NR-2"   "$2"   "$3"  0 U      0.000   0 - 0 "

#!/bin/csh
#
# Skript um aus Sparky-resonance-table eine CARA-atomlist zu machen
#
awk 'NR==1 {gsub (/h2$/,"HQ2",$2); gsub (/h5$/,"HQ5",$7); for (i=1;i<=(NF-1);i++) {printf
     "%5s",toupper($i)}} /me/ {printf "%5s%5s%5s\n","H71","H72","H73"} {if (NR!=1) {print $0"
     "$NF" "$NF}}' $1".shifts" > bla

awk 'BEGIN {COUNT=0} {if (NR==1) {for (i=1;i<=NF;i++) a[i]=$i} else {for (i=2;i<=NF;i++)
     {COUNT++; printf "%s\n",COUNT" "$i" 0.005 "a[i-1]" "NR-1}}}' bla > bla2

awk '{gsub(/g/,"GUA"); gsub(/c/,"CYT"); gsub(/a/,"ADE"); gsub(/t/,"THY"); gsub(/x/,"PUR");
     gsub(/-/,"999"); print}' bla2 > $1_cara.prot
```

The next three scripts are all needed for the NOESY back-calculation with the program GIFA (the first to modify the psf-file, the second to generate the ppm-file needed as input and the last to convert the XPLOR-NIH back-calculation output into GIFA-format.

```
   language
#!/bin/csh
#
# Dateiname des PSF-Files ohne Extension muss uebergeben werden.
# Es werden nur CYT,Thy,Ade,Gua selektiert, wenn andere
# Modifikationen vorhanden entsprechend mit einfuehren.
#
awk '{if (($1~/[0-9]+/)&&($3~/^[a-zA-Z]+$/)&&($3~/^GUA|ADE|CYT|THY|PUR$/)) {$6=".000000";
     $7="1.008"; printf "%14s%-5s%-5s%-5s%-6s%-16s%-7s%12s\n",$1"       ",$2,$3,$4,$5,$6,$7,$8;
     NR=1} else print}' $1.psf > $1"_spect.psf"
#
#
#BEGIN {getline VAR < "'"$1"'.ppm"; split (VAR,a)}
```

```
# if ($4~/^H/) { if ((a[4]==$4)&&(a[2]==$3)) {getline VAR < "'"$1"'.ppm"; split (VAR,a);
#else {$6=".000000"; $7="1.008"; printf "%14s%-5s%-5s%-5s%-6s%-16s%-7s%12s\n",$1"
     ",$2,$3,$4,$5,$6,$7,$8}} else print

#!/bin/csh
#
# Uebergabe des filenamens ohne Extension von pdb-file und shifts-file
# Viel Modifizieren nÃ¶tig, im shifts-file klein h durch goss H ersetzen
# nur A, C, T, G selektiert!!!
# in letzter awk-zeile ist die i<=Zahl anzupassen auf die Zeilenanzahl des ppm-files
awk 'NR==1 {gsub (/h2$/,"HQ2",$2); gsub (/h5$/,"HQ5",$7); for (i=1;i<=(NF-1);i++) {printf
     "%5s",toupper($i)}} /me/ {printf "%5s%5s%5s\n","H71","H72","H73"} {if (NR!=1) {print $0"
     "$NF" "$NF}}' $2"_2.shifts" > bla
awk '{if (NR==1) {for (i=1;i<=NF;i++) a[i]=$i} else {for (i=2;i<=NF;i++) printf "%s\n","PPM
     "$1" "NR-1" "a[i-1]" "$i" 1"}}' bla > bla2
awk '{gsub(/g[0-9]+/,"GUA"); gsub(/c[0-9]+/,"CYT"); gsub(/a[0-9]+/,"ADE");
     gsub(/t[0-9]+/,"THY"); gsub(/x[0-9]+/,"PUR"); print}' bla2 > bla3
awk '{if ($(NF-1)!~/^\-$/) print}' bla3 > $1.ppm
#awk '{getline VAR < "'"$1"'.pdb"; split (VAR,a); store=a[1]; if (a[1]=="ATOM") {store=a[1];
     if ((a[3]==$3)&&($4!~/\-/)) {printf "%s\n",$1" "a[4]" "$2" "$3" "$4" 1"; i=1}; getline
     VAR < "'"$1"'.pdb"; split (VAR,a)}}' bla4> $1.ppm
#awk '{if ($1=="ATOM") {if ($3!~/H/) {print} else {getline VAR < "'"$1"'.ppm"; split (VAR,a);
     for (i=1;i<=211;i++) {if (($4==a[2])&&($5==a[3])&&($3==a[4])) {print; close
     ("'"$1"'.ppm"); break} else {getline VAR < "'"$1"'.ppm"; split (VAR,a)}}; close
     ("'"$1"'.ppm")}} else print}' $1.pdb > $1_gifa.pdb
awk '{getline VAR < "'"$1"'.ppm"; split (VAR,a); for (i=1;i<=211;i++) {if
     (($2==a[2])&&($1==a[3])&&($3==a[4])) {close ("'"$1"'.ppm"); for (i=1;i<=211;i++) {if
     (($5==a[2])&&($4==a[3])&&($6==a[4])) {print "INT "$2" "$1" "$3" "$5" "$4" "$6" "$7; close
     ("'"$1"'.ppm"); break} else {getline VAR < "'"$1"'.ppm"; split (VAR,a)}} break} else
     {getline VAR < "'"$1"'.ppm"; split (VAR,a)}}; close ("'"$1"'.ppm")}' $2.spect >
     $2_gifa.spect
rm bla*

#!/bin/csh
#
# Uebergabe des .spect files ohne Extension
#
set VAR=`echo $1`
awk '{if ((NF==9)&&($3~/^(H1[^0-9a-zA-Z]|H2[^0-9a-zA-Z]*|HQ2|HQ5|H3[^0-9a-zA-Z]|H4[^0-9a-zA-Z]|
H5[^0-9a-zA-Z]*|H6|H7[0-9]|H8)$/)&&($6~/^(H1[^0-9a-zA-Z]|H2[^0-9a-zA-Z]*|HQ2|HQ5|H3[^0-9a-zA-Z]|
H4[^0-9a-zA-Z]|H5[^0-9a-zA-Z]*|H6|H7[0-9]|H8)$/)) {printf
     "%-4s%-4s%-3s%-5s%-4s%-3s%-5s%-10s\n","INT",$2,$1,$3,$5,$4,$6,$7}}' $VAR.spect >
     $VAR"_gifa.spect"
```

The next script is used to generate a latex table from a sparky shifts-file.

```
   language
#!/bin/csh
#
# Skript um aus sparky assignment tables tabellen fÃ¼r Latex zu machen
# Es wird der Dateiname des shifts-files mit extension Ã¼bergeben.
#
awk '{if (NR==1) {print "  \\arrayrulewidth0.5pt"; print "  \\doublerulesep0pt"; print "
     \\begin\{tabular\}\{c\|\|\|*\{"(NF-1)"\}\{\>\
{\\PBS\\centering\}p\{0.9cm\}\|\}\>\{\\PBS\\centering\}p\{0.9cm\}\}"; printf "%s","&"; for
     (i=1;i<=NF;i++) {if (i==NF) {printf "%s","\\textbf\{"$i"\}"; printf
```

```
"%s\n%s\n","\\\\","\\hline\\hline\\hline"} else printf "%s","\\textbf\{"$i"\}\&"}} else
{for (i=1;i<=NF;i++) {if (i==1) {printf "%s","\\textbf\{"$i"\}\&"} else {if (i==NF)
{printf "%s",$i; printf "%s\n","\\\\"} else printf "%s",$i"\&"}}}}' $1 > $1"_tab.tex"
```

The next script is used to generate and XPLOR-NIH RDC restraints file from the input to the PALES-program.

```
   language
#!/bin/csh
# used to convert pales input to xplor RDC input (with axes2.pdb and axis_500.psf)
awk '{if (NR==1) {print "! RDC table"}; if ((NR>3)&&(NF==10)) {print "assign ( resid 500  and
     name OO     )\n        ( resid 500  and name Z      )\n        ( resid 500  and name X
     )\n        ( resid 500  and name Y      )\n        ( resid "$1"      and name "$3"       )\n
           ( resid "$1"      and name "$6"       ) "$(NF-3)" "$(NF-2)" "$(NF-2)"\n\n"}}' $1 >
     $1".xplor"
```

The next two scripts are used for generating random data subsets from RDC and NOE restraints files, respectively.

```
   language
#!/bin/csh
# script to pick out every fourth (or fifth etc) NOE point from input
set VAR=`echo $1`
awk '{if ($1~/^assign/) print}' $1 > bla
awk 'BEGIN {DUMMY=5} {if (NR==DUMMY) {DUMMY=DUMMY+3; print > "'$VAR'".unused"} else print}'
     bla > $1.third
rm bla

#!/bin/csh
# script to pick out every fourth (or fifth etc) RDC point from PALES input
set VAR=`echo $1`
awk 'BEGIN {DUMMY=6;print "VARS   RESID_I RESNAME_I ATOMNAME_I RESID_J RESNAME_J ATOMNAME_J D
     DD W\nFORMAT \%5d \%6s \%6s \%5d \%6s \%6s \%9.3f \%9.3f \%.2f" >  "'$VAR'".unused"} {if
     ((NR>3)&&(NR==DUMMY)) {DUMMY=DUMMY+3; print > "'$VAR'".unused"} else print}' $1 >
     $1.third
```

The next script is used for generating a single pdb-file from the average and the 10 minimum-energy structures for submission to the PDB databank.

```
   language
#!/bin/csh
# script for generating pdb submission file with averaged,minimized structures as model 1
# and the 10 minimum-energy structures as models 2-11. TER cards, chainIDs are inserted
# and ANI residues deleted.
#
set pdbs = `awk '{if ($1~/pdb/) {printf "%s",$1" "}}' *"_##.pdb.stats"`
cat average_min.pdb $pdbs > bla
awk 'BEGIN {print "MODEL        1";COUNT1=0;COUNT2=2} {if ($1=="REMARK") print; if
     (($1=="ATOM")&&($5<=13)&&($4!="ANI")) {printf
     "%-5s%6s%5s%4s%2s%4s%12s%8s%8s%6s%6s%10s\n",$1,$2,$3,$4,"A",$5,$6,$7,$8,$9,$10,"
     ";next};if (($1=="ATOM")&&($5>=14)&&($4!="ANI")) {if (COUNT1==0) {print "TER"};printf
     "%-5s%6s%5s%4s%2s%4s%12s%8s%8s%6s%6s%10s\n",$1,$2,$3,$4,"B",$5,$6,$7,$8,$9,$10,"
     ";COUNT1=1;next}; if (($1=="END")&&(COUNT2<12)) {printf
     "%s\n%s\n%s%9s\n","TER","ENDMDL","MODEL",COUNT2;COUNT2=COUNT2+1;COUNT1=0;next}; if
```

```
    (($1=="END")&&(COUNT2==12)) {printf "%s\n%s\n%s\n","TER","ENDMDL","END"}}' bla >
    submit.pdb
#awk 'BEGIN {print "MODEL 1";COUNT1=0;COUNT2=1} {if ($1=="REMARK") print;  if
    (($1=="ATOM")&&($5<=13)&&($4!="ANI")) {printf
    "%5s%-6s%-5s%-4s%-2s%-4s%-12s%-8s%-8s%-6s%-6s%-10s\n",$1,$2,$3,$4,"A",$5,$6,$7,$8,$9,$10,"
    "}}' bla > bla2
rm bla
unset pdbs
```

The next script calls the program XPLOR-VMD with the 10 minimum-energy structures of a calculation for display.

```
  language
#!/bin/csh
# script for displaying top 10 structures of xplor-nih python script in xvmd
set pdbs = `awk '{if ($1~/pdb/) {printf "%s",$1" "}}' *"_##.pdb.stats"`
vmd-xplor $pdbs
unset pdbs
```

The next script collects the CURVES helical parameter calculation out of the 3DNA output files that were generated for the 10 minimum-energy structures of a XPLOR-NIH calculation and prints them to one file.

```
  language
#!/bin/csh
#
# Einmaliges Skript zum umnummerieren von schon vorhandenen Dateien
set i=1
while ($i<= 10)
   grep -A 17 "Curves" $i/cf_7methods.par >> comp_hel_par.tab
   @ i++
end
unset i
```

The next script selects just a subset of NOEs from an NOE input file, the rest is commented out.

```
  language
#!/bin/csh
#
# uebergabe des .tbl files ohne extension
#
awk '{if (((($6~/[hH]1.\)/)&&($11~/[hH]6\)|[hH]8\)/))
||((($6~/[hH]6\)|[hH]8\)/)&&($11~/[hH]1.\)/))||(((($6~/^[hH]2[^0-9a-zA-Z]\)$/)
&&(($11=="H6)")||($11=="H8)"))&&($3==$8))||(($6~/
[hH]2[^0-9a-zA-Z][^0-9a-zA-Z]\)/)&&(($11=="H6)")||($11=="H8)"))&&($3!=$8))))
 {if ($1=="assign") {printf "%s\n",$0} else {$1="assign"; printf "%s\n",$0}} else
     {$1="\!assign"; printf "%s\n",$0}}' $1_2.tbl > bla
awk '$1==$1 {printf "%-9s%-10s%-3s%-4s%-5s%-6s%-10s%-3s%-4s%-5s%-12s%-12s%-8s%-5s%-8s%-4s\n",
$1,$2,$3,$4,$5,$6,$7,$8,$9,$10,$11,$12,$13,$14,$15,$16}' bla > $1"_3.tbl"
rm bla*
#(($6~/[hH]6|[hH]8/)&&($11~/[hH]6|[hH]8/))||
```

Scripts for renaming atoms in files

The script is used to rename certain atoms in an input file to XPLOR-NIH calculations.

```
   language
#/!bin/csh
sort $2 > bla
sort $1.list > bla2
awk '$1!="display" {gsub("H5","HQ5)",$0);gsub("H2)","HQ2)",$0);gsub("H7)","H7#)",$0);print
     $0"\ndisplay \$result"}' bla > $2
awk '{gsub("H5)","HQ5)",$0);gsub("H2)","HQ2)",$0);gsub("H7)","H7#)",$0);print $0}' bla2 >
     $1.tbl
rm bla*
```

The script is used to rename certain atoms in a whole family of calculated structures.

```
   language
#!/bin/csh
#
#set i=1
#while ($i<= 100)
foreach i (*.pdb)
cp $i bla$i
#  sed 's/H2'\'''\'' HNF/H2bb HNF/g' $i > "bla"$i
#  sed 's/ H2'\'' HNF/H2'\'''\'' HNF/g' "bla"$i > "blabla"$i
#  sed 's/H2bb HNF/ H2'\'' HNF/g' "blabla"$i > "bla"$i
  awk '{if (($1=="REMARK")||(($2<208)||($2>235))) {print} else {if ($2==208)
       {gsub($2,$2+24);DUMMY1=$0;next};if ($2==209) {gsub($2,$2+24);DUMMY2=$0;next};if
       ($2==210) {gsub($2,$2+24);DUMMY3=$0;next};if ($2==211)
       {gsub($2,$2+24);DUMMY4=$0;next};if (($2>211)&&($2<235)) {gsub($2,$2-4);print;next};if
       ($2==235) {gsub($2,$2-4);print;print DUMMY1;print DUMMY2;print DUMMY3;print
       DUMMY4;next}}}' bla$i > $i
#  sed 's/H2'\'''\'' HNF/H2bb HNF/g' "13mer_HNF_"$i".pdb" > "bla"$i
#  sed 's/ H2'\'' HNF/H2'\'''\'' HNF/g' "bla"$i > "blabla"$i
#  sed 's/H2bb HNF/ H2'\'' HNF/g' "blabla"$i > "13mer_HNF_"$i".pdb"
#  @ i++
end
rm bla*
```

Scripts for displaying specific information content from files

This script prints out the number of intra-, interresidual and total number of NOEs, as well as the NOEs to the modification site.

```
   language
#!/bin/csh
#
# Skript gibt Anzahl der NOE, der intra- und interresidualen und der NOEs zur Modifikation
     (Achtung nur Position 7) aus
awk 'BEGIN {INTER=0;INTRA=0;MOD=0} {if ($1!="assign") {DUMMY=0} else {if ($3==$8)
     {INTRA=INTRA+1} else {if (($3==7)||($8==7)) {INTER=INTER+1;MOD=MOD+1} else
     {INTER=INTER+1}}}} END {TOTAL=INTER+INTRA;print " Total= "TOTAL;print "  intraresidual=
     "INTRA;print "  interresidual= "INTER;print " NOE to modification site = "MOD}' $1
#||($3==20)||($8==20)
```

Script code

This script prints out the Da and Rh values from all pdb-files in the working directory.

```
   language
#!/bin/csh
# script for displaying Da values of top 10 structures of xplor-nih python script
set pdbs = `awk '{if ($1~/pdb/) {printf "%s",$1" "}}' *"_##.pdb.stats"`
grep "Da:" $pdbs
unset pdbs
```

This script prints out the sorted energies from all pdb-files in the working directory.

```
   language
#!/bin/csh
grep "summary total" *.pdb | awk '{print $4" "$5" " $1}' | sort -n
```

This script prints out the sorted energies from just a subset of all pdb-files in the working directory.

```
   language
#!/bin/csh
#
# Skript um die 10 niedrigsten Energien auszugeben.
#
set i=1
grep "total" refine*.pdb* | awk '{print $5" "$1" "$2" "$3" "$4}' | sort -n | head -50 | awk
     '{print $5" "$1" "$2" "$3" "$4}' | sort -n | head -50 | awk '{print $3" "$4" "$5" "$1"
     "$2}' > 50minen
cat 50minen
while ($i<= 50)
  set VAR=`awk 'BEGIN {FS="."} {if (NR=="'$i'") printf "%s",$1}' 50minen`
  cp $VAR".pdb" "min10_"$i".pdb"
  @ i++
end
#rm bla
```

This script generates files which contain the NOE restraints information (which was used as input for the structure calculations) with the corresponding distances in the 10 minimum-energy structures. Thus not only NOE violations but also too tight NOE restraints can be easily identified.

```
   language
#!/bin/csh
#
# Skript um die 10 niedrigsten Energien auszugeben.
# Anschliessend werden die Bindungslaengen der 10 energieniedrigsten Strukturen fÃ¼r die
     noe-constraints
# hinter die eingegebenen noe-constraints ausgeschrieben.
# Es muss der Name des noe-constraint-files ohne die extension tbl angegeben werden.
# Es werden mehrere Files ausgegeben die die Auswertung der Strukturen erleichtern
#
set i=1
set VAR=`echo $1`
```

```
grep "enviol"  *.pdb | awk '{print $3" "$1" "$2" "$4" "$5}' | sort -n | awk '{print $2" "$3"
    "$1" "$4" "$5}' | head -10
grep "enviol"  *.pdb | awk '{print $3" "$1" "$2" "$4" "$5}' | sort -n | awk '{print $2" "$3"
    "$1" "$4" "$5}' | head -10 > blablabla
awk '{printf "%20s%5s%5s%5s",$3"-("$6"\/"$8"-("$11,$12,"-"$(NF-1),"+"$NF; if ($1~/^\!/)
    {printf "%5s\n","off"} else printf "%5s\n","on"}' $VAR > bla1
cat bla1 > blabla1
while ($i<= 10)
    set FILE=`awk 'BEGIN {FS="."} {if (NR=="'$i'") print $1}' blablabla`.noe
    echo $FILE
    awk '{printf "%s",$0; getline < "'$FILE'"; printf "%8.4f\n",$0}' bla$i > bonds.log
    awk '{printf "%s",$0; REF=$3; getline < "'$FILE'"; DEV=REF-$1; printf "%8.4f\n",DEV}'
        blabla$i > noedev.log
    @ i++
    mv bonds.log bla$i
    mv noedev.log blabla$i
end
awk '{printf "%s\n\n",$0}' bla$i > bonds.log
awk '{printf "%s\n\n",$0}' blabla$i > noedev.log
awk '{AVE=($(NF-9)+$(NF-8)+$(NF-7)+$(NF-6)+$(NF-5)+$(NF-4)+$(NF-3)+$(NF-2)+$(NF-1)+$NF)/10;
    RMS=((($(NF-9)-AVE)^2+($(NF-8)-AVE)^2+($(NF-7)-AVE)^2+($(NF-6)-AVE)^2+($(NF-5)-AVE)^2+
($(NF-4)-AVE)^2+($(NF-3)-AVE)^2+($(NF-2)-AVE)^2+($(NF-1)-AVE)^2+($NF-AVE)^2)/9)^(1/2);
    RMSNOE=((($(NF-9)-$3)^2+($(NF-8)-$3)^2+($(NF-7)-$3)^2+($(NF-6)-$3)^2+($(NF-5)-$3)^2+($(NF-4)-$3)^2
+($(NF-3)-$3)^2+($(NF-2)-$3)^2+($(NF-1)-$3)^2+($NF-$3)^2)/9)^(1/2); getline < "bla1"; printf
    "%s%-29s%-19s\n\n",$0,"    RMSD_AVE: "RMS,"RMSD_NOE: "RMSNOE}' bla$i > rmsd.log
rm bla*
```

Chemical shifts

In the following the chemical shifts are listed. All proton chemical shifts are referenced to the HOD signal at 4.80 ppm.

.6 Chemical shifts of 13merHNF

The chemical shifts which are unique to HNF and/or the abasic site are not listed in the table and thus are given below in ppm.
HNF (Res 7): H1" 5.32, H4 6.78, C1 109.8, C3 113.7, C4 121.2
Abasic site (Res 20): H1" 4.05

Tabelle .2: ^{1}H *chemical shifts of sugar protons of 13merHNF*

Res	H1'	H2'	H2"	H3'	H4'
G1	6,00	2,67	2,79	4,86	4,28
C2	6,09	2,14	2,54	4,85	4,27
T3	5,75	2,13	2,45	4,89	4,16
G4	5,84	2,65	2,69	5,00	4,38
C5	5,47	1,97	2,31	4,82	4,16
A6	6,34	2,82	2,78	5,06	4,40
A7	5,32	2,30	2,38	4,88	4,65
A8	6,09	2,51	2,81	4,98	4,43
C9	5,50	1,95	2,34	4,82	4,15
G10	5,96	2,62	2,78	4,96	4,37
T11	6,05	2,10	2,47	4,88	4,23
C12	5,72	2,04	2,38	4,86	4,14
G13	6,18	2,64	2,40	4,71	4,20
C14	5,72	1,85	2,36	4,70	4,07
G15	5,45	2,73	2,80	5,01	4,33
A16	6,27	2,72	2,93	5,08	4,50
C17	5,58	1,95	2,32	4,83	4,16
G18	5,94	2,60	2,76	4,98	4,37
T19	6,11	2,35	2,53	4,94	4,23
T20	4,15	2,26	2,27	4,77	4,11
T21	5,79	2,00	2,45	4,91	4,33
G22	5,75	2,58	2,66	4,97	4,33
C23	5,45	1,97	2,31	4,82	4,16
A24	6,01	2,74	2,90	5,05	4,39
G25	5,82	2,48	2,66	4,97	4,36
C26	6,10	2,15	2,22	4,47	4,05

Tabelle .3: *^{1}H chemical shifts of base protons of 13merHNF*

Res	H1	H2	H3	H41	H42	H5	H6	H7	H8
G1	-	-	-	-	-	-	-	-	7,97
C2	-	-	-	8,32	6,62	5,36	7,53	-	-
T3	-	-	13,96	-	-	-	7,34	1,64	-
G4	12,70	-	-	-	-	-	-	-	7,89
C5	-	-	-	8,21	6,40	5,43	7,37	-	-
A6	-	7,10	-	-	-	-	-	-	8,36
A7	5,97	-	6,00	-	-	7,04	7,45	-	7,71
A8	-	7,29	-	-	-	-	-	-	7,99
C9	-	-	-	7,98	6,38	5,09	7,16	-	-
G10	12,73	-	-	-	-	-	-	-	7,83
T11	-	-	13,76	-	-	-	7,29	1,41	-
C12	-	-	-	8,63	7,03	5,72	7,51	-	-
G13	-	-	-	-	-	-	-	-	7,96
C14	-	-	-	8,17	7,01	5,89	7,60	-	-
G15	12,95	-	-	-	-	-	-	-	7,96
A16	-	7,90	-	-	-	-	-	-	8,23
C17	-	-	-	8,09	6,42	5,22	7,19	-	-
G18	12,60	-	-	-	-	-	-	-	7,84
T19	-	-	13,21	-	-	-	7,29	1,50	-
T20	-	-	-	-	-	-	-	-	-
T21	-	-	12,77	-	-	-	7,29	1,51	-
G22	12,54	-	-	-	-	-	-	-	7,83
C23	-	-	-	8,26	6,35	5,38	7,35	-	-
A24	-	7,68	-	-	-	-	-	-	8,18
G25	12,93	-	-	-	-	-	-	-	7,68
C26	-	-	-	8,14	6,57	5,24	7,33	-	-

Tabelle .4: ^{13}C *chemical shifts of 13merHNF*

Res	C1'	C3'	C2	C5	C6	C8
G1	82.2	76.6	-	-	-	135.7
C2	83.9	73.9	-	95.5	140.0	-
T3	82.8	75.3	-	-	136.4	-
G4	81.4	76.5	-	-	-	135.2
C5	83.3	73.7	-	95.4	139.5	-
A6	82.3	76.7	151.3	-	-	139.1
A7	102.0	76.5 -	118.1	121.9	119.2	
A8	82.7	75.2	150.5	-	-	138.3
C9	83.1	73.8	-	95.1	139.1	-
G10	81.9	76.4	-	-	-	135.2
T11	82.7	75.0	-	-	135.8	-
C12	85.1	75.2	-	96.0	140.7	-
G13	81.8	70.5	-	-	-	136.2
C14	83.7	75.0	-	96.6	140.2	-
G15	81.2	76.6	-	-	-	135.9
A16	82.1	76.6	152.6	-	-	138.1
C17	83.1	73.7	-	95.4	138.9	-
G18	82.1	76.3	-	-	-	135.3
T19	82.7	73.7	-	-	135.8	-
T20	67.2	68.8	-	-	-	-
T21	83.5	75.2	-	-	135.9	-
G22	81.2	76.4	-	-	-	135.1
C23	83.5	73.6	-	95.4	139.9	-
A24	82.1	76.6	151.4	-	-	138.5
G25	81.2	76.3	-	-	-	134.4
C26	84.0	68.6	-	95.1	139.7	-

.7 Chemical shifts of 13merRefGC

Tabelle .5: *^{1}H chemical shifts of sugar protons of 13merRefGC*

Res	H1'	H2'	H2"	H3'	H4'		
G1	6,02	2,68	2,81	4,87	4,29	3,76	3,76
C2	6,11	2,15	2,56	4,87	4,29	4,21	4,18
T3	5,77	2,17	2,49	4,91	4,18	4,17	4,13
G4	5,88	2,65	2,72	5,01	4,39	4,08	4,15
C5	5,48	1,93	2,30	4,82	4,15	4,13	4,19
A6	5,94	2,70	2,87	5,04	4,37	4,00	4,12
A7	5,46	2,57	2,69	5,00	4,36	4,15	4,17
A8	6,18	2,60	2,89	5,02	4,45	4,15	4,21
C9	5,58	2,00	2,35	4,81	4,15	4,24	4,19
G10	5,97	2,61	2,79	4,94	4,37	4,08	4,15
T11	6,06	2,10	2,48	4,89	4,24	4,10	4,15
C12	5,73	2,04	2,39	4,86	4,14	4,08	4,10
G13	6,19	2,65	2,40	4,71	4,21	4,15	4,11
C14	5,74	1,88	2,37	4,71	4,08	3,73	3,73
G15	5,47	2,74	2,82	5,02	4,33	3,99	4,11
A16	6,28	2,73	2,94	5,08	4,50	4,19	4,25
C17	5,60	2,03	2,38	4,83	4,18	4,15	4,28
G18	5,97	2,61	2,80	4,94	4,38	4,10	4,16
T19	6,04	2,19	2,55	4,89	4,27	4,24	4,16
T20	6,00	2,11	2,51	4,81	4,19	4,24	4,15
T21	5,71	2,11	2,46	4,88	4,15	4,09	4,13
G22	5,85	2,66	2,71	4,99	4,37	4,13	4,15
C23	5,49	1,98	2,32	4,83	4,14	4,12	4,19
A24	6,01	2,74	2,89	5,05	4,39	4,01	4,13
G25	5,83	2,49	2,66	4,97	4,36	4,15	4,20
C26	6,13	2,15	2,22	4,47	4,06	4,25	4,06

Tabelle .6: *^{1}H chemical shifts of base protons of 13merRefGC*

Res	H1	H2	H3	H41	H42	H5	H6	H7	H8
G1	-	-	-	-	-	-	-	-	8,00
C2	-	-	-	8,33	6,57	5,39	7,56	-	-
T3	-	-	13,92	-	-	-	7,37	1,67	-
G4	12,92	-	-	-	-	-	-	-	7,91
C5	-	-	-	8,33	6,64	5,40	7,34	-	-
A6	-	7,52	-	-	-	-	-	-	8,15
A7	12,59	-	-	-	-	-	-	-	7,69
A8	-	7,76	-	-	-	-	-	-	8,09
C9	-	-	-	8,06	6,40	5,17	7,18	-	-
G10	12,70	-	-	-	-	-	-	-	7,82
T11	-	-	13,76	-	-	-	7,29	1,40	-
C12	-	-	-	8,61	7,00	5,72	7,52	-	-
G13	-	-	-	-	-	-	-	-	7,97
C14	-	-	-	-	-	5,91	7,62	-	-
G15	12,94	-	-	-	-	-	-	-	7,98
A16	-	7,89	-	-	-	-	-	-	8,23
C17	-	-	-	8,12	6,45	5,24	7,22	-	-
G18	12,74	-	-	-	-	-	-	-	7,82
T19	-	-	13,66	-	-	-	7,27	1,37	-
T20	-	-	-	8,33	6,89	5,60	7,59	-	-
T21	-	-	13,79	-	-	-	7,34	1,67	-
G22	12,69	-	-	-	-	-	-	-	7,90
C23	-	-	-	8,29	6,56	5,40	7,37	-	-
A24	-	7,64	-	-	-	-	-	-	8,19
G25	12,72	-	-	-	-	-	-	-	7,69
C26	-	-	-	8,30	6,57	5,32	7,38	-	-

.8 Chemical shifts of 13merRef

Tabelle .7: ^{1}H *chemical shifts of sugar protons of 13merRef*

Res	H1'	H2'	H2"	H3'	H4'
G1	6.02	2.68	2.81	4.87	4.28
C2	6.11	2.15	2.55	4.86	4.28
T3	5.76	2.15	2.47	4.90	4.17
G4	5.86	2.63	2.70	4.99	4.38
C5	5.41	1.84	2.22	4.79	4.11
A6	5.75	2.71	2.84	5.03	4.36
A7	5.84	2.63	2.84	5.05	4.43
A8	6.05	2.54	2.83	5.00	4.44
C9	5.56	1.92	2.32	4.77	4.14
G10	5.95	2.61	2.78	4.95	4.36
T11	6.05	2.10	2.47	4.88	4.23
C12	5.72	2.03	2.38	4.85	4.14
G13	6.18	2.64	2.40	4.70	4.20
C14	5.73	1.87	2.37	4.70	4.07
G15	5.46	2.73	2.80	5.01	4.33
A16	6.27	2.71	2.92	5.08	4.49
C17	5.56	1.99	2.36	4.82	4.17
G18	6.00	2.62	2.82	4.96	4.39
T19	6.04	2.14	2.61	4.87	4.28
T20	6.13	2.17	2.62	4.91	4.19
T21	5.84	2.10	2.48	4.91	4.15
G22	5.82	2.65	2.67	5.00	4.38
C23	5.49	1.98	2.32	4.83	4.16
A24	6.01	2.74	2.89	5.04	4.38
G25	5.82	2.48	2.66	4.96	4.36
C26	6.13	2.14	2.20	4.47	4.05

Tabelle .8: ^{1}H *chemical shifts of base protons of 13merRef*

Res	H1	H2	H3	H41	H42	H5	H6	H7	H8
G1	-	-	-	-	-	-	-	-	7.99
C2	-	-	-	8.33	6.62	5.39	7.55	-	-
T3	-	-	13.95	-	-	-	7.36	1.66	-
G4	12.71	-	-	-	-	-	-	-	7.89
C5	-	-	-	8.34	6.31	5.40	7.30	-	-
A6	-	7.17	-	-	-	-	-	-	8.19
A7	-	7.13	-	-	-	-	-	-	8.11
A8	-	7.58	-	-	-	-	-	-	8.05
C9	-	-	-	7.91	6.36	5.10	7.12	-	-
G10	12.67	-	-	-	-	-	-	-	7.81
T11	-	-	13.75	-	-	-	7.27	1.39	-
C12	-	-	-	8.62	7.02	5.72	7.51	-	-
G13	-	-	-	-	-	-	-	-	7.97
C14	-	-	-	-	-	5.91	7.62	-	-
G15	12.95	-	-	-	-	-	-	-	7.97
A16	-	7.91	-	-	-	-	-	-	8.23
C17	-	-	-	8.11	6.44	5.23	7.21	-	-
G18	12.76	-	-	-	-	-	-	-	7.83
T19	-	-	13.91	-	-	-	7.25	1.37	-
T20	-	-	13.90	-	-	-	7.47	1.62	-
T21	-	-	13.70	-	-	-	7.33	1.69	-
G22	12.60	-	-	-	-	-	-	-	7.89
C23	-	-	-	8.32	6.34	5.39	7.37	-	-
A24	-	7.66	-	-	-	-	-	-	8.19
G25	12.94	-	-	-	-	-	-	-	7.69
C26	-	-	-	-	-	5.33	7.38	-	-

Tabelle .9: *^{13}C chemical shifts of 13merRef*

Res	C1'	C3'	C2	C5	C6	C8
G1	82.2	75.3	-	-	-	135.9
C2	83.9	76.6	-	95.7	140.1	-
T3	82.8	75.3	-	-	136.6	-
G4	81.4	76.5	-	-	-	135.5
C5	83.3	76.1	-	95.5	139.6	-
A6	81.7	76.9	151.4	-	-	138.7
A7	82.2	76.1	151.1	-	-	138.2
A8	81.9	76.6	152.0	-	-	138.0
C9	83.1	74.0	-	95.1	139.1	-
G10	82.0	76.9	-	-	-	135.5
T11	82.7	75.1	-	-	135.8	-
C12	83.8	76.5	-	96.1	141.0	-
G13	82.0	70.6	-	-	-	135.5
C14	85.2	75.1	-	96.8	140.4	-
G15	81.3	76.7	-	-	-	136.4
A16	82.1	76.9	152.7	-	-	138.4
C17	83.2	74.0	-	95.4	139.2	-
G18	82.1	75.2	-	-	-	135.5
T19	82.7	75.1	-	-	135.8	-
T20	82.7	74.8	-	-	137.2	-
T21	81.5	74.7	-	-	136.7	-
G22	81.3	76.6	-	-	-	135.5
C23	83.5	75.3	-	95.5	139.9	-
A24	82.1	77.0	151.5	-	-	138.8
G25	81.2	75.0	-	-	-	134.5
C26	84.0	68.8	-	95.3	140.2	-

.9 Chemical shifts of 13mer2AP

Tabelle .10: ^{1}H *chemical shifts of sugar protons of 13mer2AP*

Res	H1'	H2'	H2"	H3'	H4'
G1	6.02	2.68	2.81	4.88	4.30
C2	6.11	2.15	2.55	4.88	4.29
T3	5.77	2.15	2.48	4.91	4.17
G4	5.87	2.66	2.72	5.01	4.39
C5	5.49	1.92	2.29	4.83	4.15
A6	5.92	2.70	2.89	5.05	4.36
X7	5.55	2.57	2.70	5.01	4.38
A8	6.17	2.60	2.89	5.03	4.47
C9	5.56	1.97	2.34	4.83	4.14
G10	5.96	2.63	2.79	4.96	4.38
T11	6.06	2.11	2.49	4.89	4.24
C12	5.73	2.05	2.40	4.87	4.15
G13	6.19	2.66	2.42	4.72	4.22
C14	5.73	1.87	2.38	4.71	4.09
G15	5.47	2.74	2.82	5.03	4.34
A16	6.29	2.74	2.95	5.09	4.51
C17	5.60	2.03	2.38	4.85	4.19
G18	6.00	2.66	2.82	4.98	4.40
T19	6.07	2.14	2.64	4.89	4.27
T20	6.05	2.21	2.54	4.89	4.21
T21	5.73	2.14	2.47	4.91	4.15
G22	5.86	2.64	2.72	5.00	4.38
C23	5.48	1.97	2.32	4.84	4.15
A24	6.01	2.75	2.90	5.05	4.39
G25	5.83	2.49	2.66	4.98	4.37
C26	6.12	2.16	2.23	4.48	4.06

Tabelle .11: ^{1}H *chemical shifts of base protons of 13mer2AP*

Res	H1	H2	H3	H41	H42	H5	H6	H7	H8
G1	-	-	-	-	-	-	-	-	7.99
C2	-	-	-	8.33	6.62	5.38	7.55	-	-
T3	-	-	13.95	-	-	-	7.36	1.68	-
G4	12.72	-	-	-	-	-	-	-	7.92
C5	-	-	-	8.30	6.32	5.42	7.34	-	-
A6	-	7.33	-	-	-	-	-	-	8.20
X7	-	-	-	-	-	-	7.80	-	7.95
A8	-	7.72	-	-	-	-	-	-	8.09
C9	-	-	-	8.02	6.40	5.16	7.15	-	-
G10	12.72	-	-	-	-	-	-	-	7.83
T11	-	-	13.77	-	-	-	7.29	1.42	-
C12	-	-	-	8.64	7.04	5.73	7.52	-	-
G13	-	-	-	-	-	-	-	-	7.97
C14	-	-	-	-	-	5.90	7.62	-	-
G15	12.97	-	-	-	-	-	-	-	7.98
A16	-	7.92	-	-	-	-	-	-	8.24
C17	-	-	-	8.11	6.46	5.25	7.23	-	-
G18	12.72	-	-	-	-	-	-	-	7.86
T19	-	-	13.69	-	-	-	7.29	1.41	-
T20	-	-	13.38	-	-	-	7.46	1.65	-
T21	-	-	13.57	-	-	-	7.34	1.69	-
G22	12.66	-	-	-	-	-	-	-	7.89
C23	-	-	-	8.34	6.35	5.40	7.36	-	-
A24	-	7.68	-	-	-	-	-	-	8.19
G25	12.94	-	-	-	-	-	-	-	7.69
C26	-	-	-	-	-	5.26	7.35	-	-

Tabelle .12: ^{13}C *chemical shifts of 13mer2AP*

Res	C1'	C3'	C2	C5	C6	C8
G1	82.2	73.1	-	-	-	134.6
C2	83.9	76.5	-	95.5	140.1	-
T3	82.8	75.3	-	-	136.6	-
G4	81.4	76.5	-	-	-	135.5
C5	83.2	76.1	-	95.5	139.7	-
A6	81.7	76.8	151.5	-	-	138.7
X7	82.2	76.9	-	-	147.6	138.2
A8	81.9	76.6	152.0	-	-	138.1
C9	83.1	74.0	-	95.1	139.1	-
G10	81.9	76.7	-	-	-	135.5
T11	82.7	75.1	-	-	135.8	-
C12	85.1	76.5	-	96.0	141.1	-
G13	82.0	70.5	-	-	-	135.5
C14	83.7	74.9	-	96.8	140.4	-
G15	81.2	76.7	-	-	-	136.4
A16	82.1	76.9	152.7	-	-	138.4
C17	83.2	74.0	-	95.2	139.3	-
G18	82.1	75.3	-	-	-	135.5
T19	82.7	75.1	-	-	135.8	-
T20	82.5	74.5	-	-	137.2	-
T21	81.4	74.4	-	-	136.7	-
G22	81.2	76.6	-	-	-	135.5
C23	83.4	74.0	-	95.5	139.9	-
A24	82.1	76.1	151.5	-	-	138.8
G25	81.2	75.3	-	-	-	134.4
C26	84.0	68.6	-	95.4	139.9	-

.10 Chemical shift differences for 13merRef and 13mer2AP

The chemical shift differences are calculated as X(13merRef)-X(13mer2AP).

Tabelle .13: ^{1}*H chemical shift differences between 13merRef and 13mer2AP, for X7 H2 the difference the CSD between atoms H2 in 13merRef and H6 in 13mer2AP is given.*

Res	H1'	H2'	H2"	H3'	H4'	H1/H3 H41/H42	H2/H5 H7	H6/H8
G1	0.00	0.00	0.00	-0.01	-0.02	-	-	0.00
C2	-0.01	0.00	0.00	-0.01	-0.01	-0.01/0.00	0.00	0.00
T3	-0.01	0.00	-0.01	-0.01	-0.01	0.00	-0.01	0.00
G4	-0.01	-0.03	-0.02	-0.02	-0.02	-0.01	-	-0.03
C5	-0.08	-0.08	-0.07	-0.04	-0.04	0.03/-0.01	-0.02	-0.04
A6	-0.17	0.01	-0.05	-0.02	-0.01	-	-0.16	-0.01
X7	0.29	0.06	0.14	0.04	0.05	-	-0.67	0.16
A8	-0.12	-0.06	-0.06	-0.03	-0.03	-	-0.13	-0.04
C9	0.00	-0.05	-0.02	-0.06	0.00	-0.03/0.02	-0.06	-0.04
G10	-0.01	-0.01	-0.02	-0.01	-0.02	-0.05	-	-0.02
T11	-0.01	-0.01	-0.01	-0.01	-0.01	-0.02	-0.03	-0.02
C12	-0.01	-0.01	-0.01	-0.02	-0.01	-0.02/-0.01	-0.01	-0.01
G13	-0.01	-0.02	-0.02	-0.02	-0.01	-	-	-0.01
C14	0.00	0.00	-0.01	-0.01	-0.02	-	0.01	0.00
G15	-0.01	-0.01	-0.02	-0.02	-0.01	-0.01	-	-0.01
A16	-0.02	-0.02	-0.02	-0.02	-0.02	-	-0.01	-0.02
C17	-0.04	-0.04	-0.02	-0.03	-0.02	-0.01/-0.02	-0.02	-0.02
G18	0.00	-0.03	0.00	-0.02	-0.01	0.03	-	-0.03
T19	-0.03	0.00	-0.03	-0.01	0.01	0.21	-0.04	-0.04
T20	0.09	-0.04	0.09	0.02	-0.03	0.53	-0.03	0.01
T21	0.11	-0.04	0.01	0.01	0.01	0.13	0.00	-0.02
G22	-0.04	0.00	-0.05	0.00	0.00	-0.05	-	0.00
C23	0.01	0.01	0.01	-0.01	0.01	-0.02/-0.02	-0.02	0.01
A24	0.00	-0.01	-0.01	-0.01	-0.01	-	-0.02	-0.01
G25	-0.01	-0.01	-0.01	-0.02	-0.01	0.00	-	0.00
C26	0.01	-0.02	-0.02	-0.01	0.00	-	0.07	0.03

Helical parameter

The averaged values for the 10 minimum-energy, violation-free structures for all helical parameters are listed in the Appendix in sections .11 and .12 for 13merRef and 13mer2AP, respectively. Their RMSD is given as the uncertainty.

.11 Helical parameters for 13merRef

Tabelle .14: *Base pair parameters for 13merRef, translational*

base pair	Shear (Sx)/Å	Stretch (Sy)/Å	Stagger (Sz)/Å
G1–C26	-0,42 ± 0,01	-0,26 ± 0,00	-0,14 ± 0,04
C2–G25	0,42 ± 0,02	-0,26 ± 0,01	-0,13 ± 0,05
T3–A24	-0,07 ± 0,01	-0,26 ± 0,01	0,07 ± 0,04
G4–C23	-0,37 ± 0,01	-0,28 ± 0,00	0,10 ± 0,06
C5–G22	0,40 ± 0,01	-0,26 ± 0,01	-0,13 ± 0,05
A6–T21	0,02 ± 0,02	-0,26 ± 0,01	-0,04 ± 0,03
A7–T20	0,07 ± 0,01	-0,27 ± 0,01	-0,24 ± 0,02
A8–T19	0,03 ± 0,02	-0,28 ± 0,01	-0,06 ± 0,04
C9–G18	0,39 ± 0,01	-0,27 ± 0,00	-0,05 ± 0,07
G10–C17	-0,40 ± 0,01	-0,26 ± 0,00	0,08 ± 0,03
T11–A16	-0,07 ± 0,00	-0,27 ± 0,01	0,09 ± 0,07
C12–G15	0,40 ± 0,01	-0,27 ± 0,00	-0,11 ± 0,05
G13–C14	-0,40 ± 0,01	-0,26 ± 0,00	-0,03 ± 0,02
average	0.00 ± 0.33	-0.26 ± 0.01	-0.05 ± 0.11

Tabelle .15: *Base pair parameters for 13merRef, rotational*

base pair	Buckle (χ)/°	Propeller Twist (ω)/°	Opening (σ)/°
G1–C26	-1,4 ± 1,5	2,4 ± 0,6	1,7 ± 0,1
C2–G25	-4,7 ± 1,9	3,8 ± 1,7	1,5 ± 0,2
T3–A24	-8,0 ± 1,7	-1,8 ± 1,7	-2,9 ± 0,1
G4–C23	-0,4 ± 0,5	5,4 ± 2,5	1,5 ± 0,1
C5–G22	5,3 ± 0,5	3,5 ± 1,7	1,6 ± 0,1
A6–T21	6,3 ± 0,4	-1,8 ± 0,4	-2,9 ± 0,2
A7–T20	4,9 ± 0,4	-8,2 ± 1,3	-2,5 ± 0,3
A8–T19	5,1 ± 0,7	-10,6 ± 1,0	-2,7 ± 0,3
C9–G18	4,3 ± 1,0	-6,0 ± 1,2	1,5 ± 0,1
G10–C17	-1,2 ± 1,3	1,2 ± 1,8	1,5 ± 0,1
T11–A16	2,6 ± 2,7	-3,4 ± 1,4	-3,0 ± 0,2
C12–G15	4,6 ± 1,6	-8,9 ± 0,6	2,0 ± 0,1
G13–C14	-0,6 ± 0,8	-0,6 ± 0,5	1,4 ± 0,1
average	1.3 ± 4.4	-1.9 ± 5.2	-0.1 ± 2.2

Tabelle .16: *Base pair step parameters for 13merRef, translational*

base pair step	Shift (Sx)/Å	Slide (Sy)/Å	Rise (Sz)/Å
G1–C2	-0,12 ± 0,03	-0,71 ± 0,11	3,40 ± 0,07
C2–T3	-0,30 ± 0,05	-1,35 ± 0,04	3,38 ± 0,04
T3–G4	0,44 ± 0,05	-0,57 ± 0,11	2,81 ± 0,06
G4–C5	0,10 ± 0,05	-0,25 ± 0,10	3,06 ± 0,05
C5–A6	-0,61 ± 0,11	-0,67 ± 0,06	3,01 ± 0,03
A6–A7	-0,36 ± 0,03	-0,87 ± 0,02	3,27 ± 0,01
A7–A8	-0,45 ± 0,07	-0,66 ± 0,06	3,11 ± 0,03
A8–C9	-0,02 ± 0,08	-0,72 ± 0,08	3,20 ± 0,01
C9–G10	0,17 ± 0,07	-0,98 ± 0,09	3,21 ± 0,05
G10–T11	-0,23 ± 0,03	-0,92 ± 0,04	3,14 ± 0,03
T11–C12	0,34 ± 0,10	-0,28 ± 0,09	3,00 ± 0,06
C12–G13	0,02 ± 0,04	-0,99 ± 0,05	3,01 ± 0,04
average	-0.08 ± 0.32	-0.75 ± 0.31	3.13 ± 0.17

Tabelle .17: *Base pair step parameters for 13merRef, rotational*

base pair step	Tilt (τ)/°	Roll (ρ)/°	Twist (Ω)/°
G1–C2	-1,0 ± 0,4	-4,9 ± 1,0	41,1 ± 0,6
C2–T3	1,6 ± 0,3	-2,6 ± 1,3	30,4 ± 0,3
T3–G4	-0,6 ± 0,3	3,7 ± 0,9	35,2 ± 0,6
G4–C5	2,1 ± 0,1	2,1 ± 0,8	40,6 ± 0,6
C5–A6	-1,1 ± 0,2	4,6 ± 0,4	35,2 ± 0,4
A6–A7	0,3 ± 0,2	-2,4 ± 0,6	33,4 ± 0,2
A7–A8	-4,2 ± 0,3	1,3 ± 0,3	36,0 ± 0,5
A8–C9	-2,7 ± 0,5	-2,8 ± 0,6	37,6 ± 0,6
C9–G10	-2,3 ± 0,4	0,7 ± 1,2	33,3 ± 0,8
G10–T11	1,8 ± 0,3	-3,0 ± 0,6	35,3 ± 0,6
T11–C12	3,3 ± 0,5	2,7 ± 1,4	39,6 ± 0,7
C12–G13	-1,0 ± 0,5	10,0 ± 1,3	30,6 ± 0,5
average	-0.3 ± 2.2	0.8 ± 4.2	35.7 ± 3.5

.12 Helical parameters for 13mer2AP

Tabelle .18: *Base pair parameters for 13mer2AP, translational*

base pair	Shear (Sx)/Å	Stretch (Sy)/Å	Stagger (Sz)/Å
G1–C26	-0,40 ± 0,01	-0,26 ± 0,00	-0,11 ± 0,04
C2–G25	0,35 ± 0,02	-0,28 ± 0,01	-0,10 ± 0,05
T3–A24	-0,07 ± 0,00	-0,26 ± 0,00	-0,02 ± 0,05
G4–C23	-0,37 ± 0,01	-0,29 ± 0,00	0,33 ± 0,03
C5–G22	0,29 ± 0,03	-0,27 ± 0,01	-0,10 ± 0,05
A6–T21	0,01 ± 0,02	-0,27 ± 0,00	-0,02 ± 0,04
2AP7–T20	-0,02 ± 0,00	-0,32 ± 0,00	-0,23 ± 0,03
A8–T19	0,08 ± 0,01	-0,26 ± 0,00	0,12 ± 0,03
C9–G18	0,43 ± 0,02	-0,25 ± 0,01	-0,18 ± 0,06
G10–C17	-0,39 ± 0,02	-0,27 ± 0,00	-0,16 ± 0,03
T11–A16	-0,07 ± 0,01	-0,26 ± 0,00	-0,13 ± 0,05
C12–G15	0,41 ± 0,01	-0,27 ± 0,01	-0,17 ± 0,04
G13–C14	-0,37 ± 0,02	-0,26 ± 0,01	-0,05 ± 0,03
average	-0.01 ± 0.31	-0.27 ± 0.02	-0.06 ± 0.15

Tabelle .19: *Base pair parameters for 13mer2AP, rotational*

base pair	Buckle (χ)/°	Propeller Twist (ω)/°	Opening (σ)/°
G1–C26	-2,2 ± 1,2	2,1 ± 0,4	1,5 ± 0,1
C2–G25	-3,0 ± 1,4	6,0 ± 2,0	1,0 ± 0,1
T3–A24	-5,8 ± 1,5	-2,4 ± 1,3	-2,8 ± 0,1
G4–C23	5,7 ± 0,7	6,9 ± 1,6	1,8 ± 0,2
C5–G22	8,0 ± 0,6	1,9 ± 1,0	0,9 ± 0,1
A6–T21	6,4 ± 0,3	-4,9 ± 0,3	-2,8 ± 0,1
2AP7–T20	2,3 ± 0,3	-1,2 ± 0,3	-5,7 ± 0,1
A8–T19	5,3 ± 0,7	-2,5 ± 1,1	-3,0 ± 0,1
C9–G18	8,4 ± 0,7	-3,7 ± 1,1	1,8 ± 0,2
G10–C17	-0,5 ± 1,3	6,2 ± 1,5	1,3 ± 0,2
T11–A16	2,8 ± 2,2	0,8 ± 1,0	-2,9 ± 0,1
C12–G15	2,7 ± 1,4	-6,5 ± 1,1	2,1 ± 0,1
G13–C14	-2,1 ± 1,5	-1,0 ± 1,0	1,3 ± 0,1
average	2.1 ± 4.5	0.1 ± 4.3	-0.4 ± 2.6

Tabelle .20: *Base pair step parameters for 13mer2AP, translational*

base pair step	Shift (Sx)/Å	Slide (Sy)/Å	Rise (Sz)/Å
G1–C2	-0,03 ± 0,07	-0,44 ± 0,11	3,28 ± 0,05
C2–T3	-0,04 ± 0,05	-1,25 ± 0,04	3,28 ± 0,05
T3–G4	0,28 ± 0,02	-0,85 ± 0,04	2,73 ± 0,06
G4–C5	0,06 ± 0,03	-0,29 ± 0,05	3,16 ± 0,03
C5–A6	-0,43 ± 0,03	-0,73 ± 0,05	3,07 ± 0,04
A6–2AP7	-0,58 ± 0,02	-1,07 ± 0,07	3,31 ± 0,02
2AP7–A8	-0,13 ± 0,03	-0,66 ± 0,04	3,14 ± 0,02
A8–C9	-0,10 ± 0,05	-0,98 ± 0,05	3,18 ± 0,02
C9–G10	0,11 ± 0,04	-1,10 ± 0,05	3,29 ± 0,05
G10–T11	0,06 ± 0,05	-0,87 ± 0,06	3,29 ± 0,03
T11–C12	0,31 ± 0,07	-0,39 ± 0,13	3,10 ± 0,04
C12–G13	-0,09 ± 0,09	-0,99 ± 0,08	3,01 ± 0,07
average	-0.05 ± 0.26	-0.80 ± 0.30	3.15 ± 0.17

Tabelle .21: *Base pair step parameters for 13mer2AP, rotational*

base pair step	Tilt (τ)/°	Roll (ρ)/°	Twist (Ω)/°
G1–C2	-1,1 ± 0,5	-2,3 ± 1,5	42,3 ± 0,8
C2–T3	2,3 ± 0,6	-1,1 ± 1,4	31,1 ± 0,4
T3–G4	-3,0 ± 0,3	-0,5 ± 0,5	34,0 ± 0,5
G4–C5	4,1 ± 0,3	0,7 ± 0,9	41,3 ± 0,4
C5–A6	-1,0 ± 0,2	2,5 ± 0,4	35,5 ± 0,5
A6–2AP7	-2,1 ± 0,1	0,4 ± 0,3	34,0 ± 0,4
2AP7–A8	-5,3 ± 0,3	-3,3 ± 0,3	37,6 ± 0,3
A8–C9	-0,3 ± 0,4	-3,0 ± 0,5	34,1 ± 0,5
C9–G10	-1,5 ± 0,3	3,9 ± 0,9	32,9 ± 0,5
G10–T11	3,3 ± 0,4	-5,5 ± 0,6	35,8 ± 0,3
T11–C12	3,0 ± 0,4	4,3 ± 1,6	39,1 ± 1,0
C12–G13	-0,8 ± 0,3	10,7 ± 1,6	30,6 ± 0,6
average	-0.2 ± 2.8	0.6 ± 4.3	35.7 ± 3.7

List of abbreviations

WC **W**atson-**C**rick

DNA **D**esoxyribo**N**ucleic **A**cid

NMR **N**uclear **M**agnetic **R**esonance

SNP **S**ingle **N**nucleotide **P**olymorphism

DETEQ **D**etection by **E**lectron **T**ransfer controlled **E**mission **Q**uenching

FIT **F**orced **I**ntercala**T**ion probe

FRET **F**luorescence **R**esonance **E**xcitation **T**ransfer

2AP **2**-**A**mino**p**urin

HNF 2-**H**ydroxy-7-**n**itro**f**luorene

ABA **ABA**sic site

A **A**denine

T **T**hymine

G **G**uanine

C **C**ytosine

TRIS **Tris**hydroxymethylaminomethane

NOE **N**uclear **O**verhauser **E**nhancement

NOESY **N**uclear **O**verhauser **E**nhancement **S**pectroscop**y**

ISPA **I**solated **S**pin **P**air **A**pproximation

RDC **R**esidual **D**ipolar **C**oupling

PAS **P**rinciple **A**xis **S**ystem

SA **S**imulated **A**nnealing

MD **M**olecular **D**ynamics

HPLC **H**igh **P**ressure **L**iquid **C**hromatography

WATERGATE **W**ater suppression by **GrA**dient **T**ailored **E**xcitation

DQF-COSY **D**ouble **Q**uantum **F**iltered **CO**rrelated **S**pectroscop**y**

TOCSY **TO**tal **C**orrelation **S**pectroscop**y**

HMQC **H**eteronuclear **M**ultiple **Q**uantum **C**oherence Spectroscopy

DFT **D**ensity **F**unctional **T**heory

TZVP **T**riple **Z**eta **V**alence plus **P**olarization

MEP **M**olecular **E**lectrostatic **P**otential

P.E.-COSY **P**rimitive **E**xclusive **CO**rrelated **S**pectroscop**y**

RMSD **R**oot **M**ean **S**quare **D**eviation

CSD **C**hemical **S**hift **D**ifference

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Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe.

Berlin, den 28.01.2008 André Dallmann